IMMUNOFLUORESCENT CHARACTERIZATION OF COLLAGEN TYPES I AND III IN NORMAL SAPHENOUS VEINS AND AORTOCORONARY SAPHENOUS VEIN GRAFTS

JONATHAN KOLITZ

1979

Permission for photocopying or microfilming of "Immunoflourescent Characterization of Types I and III Collagen in Normal Saphenous Veins and Aortocoronary Saphenous Vein Bypass G[illegible]"

(TITLE OF THESIS)

for the purpose of individual scholarly consultation or reference is hereby granted by the author. This permission is not to be interpreted as affecting publication of this work or otherwise placing it in the public domain, and the author reserves all rights of ownership guaranteed under common law protection of unpublished manuscripts.

Jonathan Kolif

Signature of Author

May 11, 1978

Date

IMMUNOFLUORESCENT CHARACTERIZATION OF COLLAGEN TYPES I AND III IN NORMAL SAPHENOUS VEINS AND AORTOCORONARY SAPHENOUS VEIN GRAFTS

Jonathan Kolitz

A Thesis Submitted to the

Yale University School of Medicine

in Partial Fulfillment of the Requirement

for the Degree of Doctor of Medicine

1979

ACKNOWLEDGMENTS

I wish to thank Drs. Joseph Madri, Graeme Hammond and Joseph Roll and Mr. Frederick Putt for the help they extended to me during this work. Dr. Vincent Marchesi was especially helpful, and provided guidance and support for which I am grateful.

TABLE OF CONTENTS

SECTION I: INTRODUCTION

A. NORMAL VENOUS STRUCTURE

Before proceeding to explore selected aspects of venous pathology in the setting of venous hypertension, it is appropriate to review the normal microscopic architecture of veins. Excellent summaries of venous histology (Rhodin, 1974; Ham, 1974; Bloom and Fawcett, 1975) as well as more extensive treatises (Franklin, 1937) have been published.

Arteries were first distinguished from veins by Praxagoras of Cos in the Fourth Century B.C. By the Third Century B.C., the fact that arteries tend to pulsate, while veins do not, had been appreciated. Aristotle was of the opinion that blood was contained only in the veins, the liver, and the right side of the heart, the arteries serving solely as the conduit for the "vital spirit," which was derived from transformed air in the lungs. By the Second Century, Galen propounded the view, held for the next fifteen hundred years, that the veins originate from the liver and communicate with the arterial system by means of minute terminal anastamoses, as well as foramina putatively located in the interventricular septum.

The multilayered nature of the venous wall was also first alluded to by Galen, who felt that veins usually have one coat, except in areas necessitating firm external support, where an additional, external coat might be present. A clearer distinction between media (along with the unsuspected intima) and adventitia emerges from the work of Vesalius. He distinguished between an "essential" layer, containing

muscle fibers distinct from those found in skeletal muscle, and a looser, outer layer which he thought was derived from surrounding structures and which became progressively attenuated as veins penetrated parenchymal organs.

Charles Estienne first observed the venous valves in the mid Sixteenth Century. His work and that of other workers later in that century, particularly the studies of Fabricius ab Aquapendente, greatly influenced Harvey in finally establishing the directionality of blood flow and the nature of the circulation. It was the understanding of the blood's circulation that established and, indeed, defined the veins as directional units coursing at all times in the direction of the heart. Over the ensuing centuries, our understanding of the veins has become increasingly refined, with the venous system emerging not only as a passive conduit, but as an active participant in the complex web of neurohumoral integrative mechanisms which underlies the cardiac output. The postcapillary venule, the venous system's smallest component, has taken on increasing importance as the key functional unit of the inflammatory response.

Unlike the arterial system, strict correspondence between venous ultrastructure and caliber tends not to prevail. In his classic Monograph on Veins, Franklin (1937) felt that full justice could be done to the named veins only by means of exhaustive individual descriptions. Certain generalizations are possible, however.

The vein wall, like that of the arteries, has three layers -- tunica intima, tunica media, and tunica adventitia. These layers are not as distinct in veins as they are in arteries. This is partly

due to the paucity of elastic fibers in veins which prevents, in most instances, a clear demarcation of the layers by means of well-organized internal and external elastic membranes. In general, veins possess less smooth muscle and more collagen than arteries (Ludbrook, 1966).

The tunica intima consists of a thin layer of continuous endothelium resting on variable quantities of connective tissue. The subendothelial connective tissue tends to be only moderately developed, even in large veins. The normal human portal vein has been found to be actually devoid of a subendothelium (Li, 1940; Wilson, 1961), while an age related increase in the thickness of the subendothelium has been noted in the human inferior vena cava (Stuart and Magarey, 1960) and saphenous vein (Lawrie et al., 1976). Increases in intimal thickness is one of the principal features of venous pathology. This has taken a considerable amount of time to be appreciated. Wagner and Herbut (1949) illustrate the normal structure of the saphenous vein with a photomicrograph showing marked intimal proliferation.

A more refined understanding of intimal structure has come from the application of freeze-fracture methods (Simionescu et al., 1975 and 1976). Using veins from rat omentum and mesentery, the intima of pericytic venules (20 μm to 50 μm in diameter) was seen to be interconnected by a scattered array of complexes interpreted to be tight junctions. Gap junctions are first seen in association with tight junctions in muscular venules (50 μm to 200 μm in diameter). Similar but less discontinuous junctions are seen in the larger veins, interspersed with long, continuous tight junctions. These elements are

similar, but less elaborate than those present in arteries.

The emergence of the tunica media as a distinct component of the vein wall was traced in subdermal vessels of rabbit skin by Rhodin (1969). Fibroblasts and pericytes (the latter are generally felt to be similar to fibroblasts, but have a more granular rough endoplasmic reticulum and, unlike fibroblasts, possess a basement membrane -- these cells may be primitive smooth muscle cell precursors) first appear in post-capillary (pericytic) venules, and are present in increasing numbers with increases in vessel diameter. Collecting venules have one complete layer of pericytes external to the tunica intima, with an outer layer of cells described as fibroblasts. Occasional primitive smooth muscle cells (SMCs) are seen in muscular venules and, at the level of small collecting veins, a prominent media is seen, with several continuous SMC layers. Throughout this progression, the ratio of luminal diameter to wall thickness actually increases, from 10 to 1 in post-capillary venules to 100 to 1 in small collecting veins.

As veins increase in caliber, variations in the structure of the media become more apparent. In human kidney and spleen, venules larger than 300 μm in diameter may still have discontinuous SMC layers in the media. In large veins, particularly the inferior vena cava, the media may be so inconspicuous that longitudinally oriented adventitial SMC bundles may appear to be abutting directly on the intima. The veins of the maternal placenta, nail bed, retina, bond, brain, and meninges are generally devoid of SMC fibers, while the veins of the pregnant uterus, umbilical cord, and adrenal glands are richly supplied

with them (Payan and Gilbert, 1972).

In general, the media of veins which receive substantial external support, e.g., those which are intramuscular and intraabdominal, have a thinner, less muscular media than those which do not. The veins of the lower extremity have significantly more smooth muscle than upper extremity veins. The arrangement of the SMCs also varies with location, with venous medial SMCs disposed primarily longitudinally in the thorax and abdomen, and circularly in the extremities. Longitudinally oriented fibers prevent the collapse of vessels of low internal pressure (Gross and Handler, 1939), while circular fibers facilitate the capacitance and pumping functions of the veins of the extremities.

An interesting variation of venous medial structure is seen in the mammalian portal vein (Ts'ao, Glagov and Kelsey, 1971). The media of the extrahepatic segment of this vein consists of two mutually perpendicular layers of SMCs, separated by an acellular fibrous layer. The inner zone is avascular, with circumferentially oriented SMCs. The outer zone is thicker, has longitudinally arranged SMCs, and is well vascularized. It is speculated that this arrangement may subserve a sphincteric role in mediating between the portal flow and hepatic vacular resistance.

In the neurovascular complex entering the human kidney, a unique arrangement of medial smooth muscle has been demonstrated (Payan and Gilbert, 1972). Eccentric longitudinal muscle fibers were seen to extend from the vein wall to the parenchyma of the kidney, occasionally extending from one vein to another. Their function remains to be clarified.

The adventitia of veins is considerably thicker than the media. It consists of a network of loose connective tissue containing thick, longitudinally oriented bundles of collagen and elastin. Occasionally, longitudinally disposed SMCs may be present adjacent to the media. Circularly oriented cardiac muscle fibers are found in the adventitia of the pulmonary veins and venae cavae as they enter the heart. It is through the adventitia that the vessels which nourish the veins course.

Sections treated with ortholidine or benzidine have been used to demarcate the course of the vasa venorum in dog and man (Brook, 1977). Arterial (6.2 to 6.6 μm in diameter) and venous (9.0 to 133 μm) vasa were shown to be running together in groups of two and three. The venous vasa are more tortuous and have more branch points than arterial vasa. The caliber of both types of vasa becomes increasingly attenuated as they course from adventitia into media. While vasa were seen adjacent to the intima in the human long saphenous vein, no direct communication with the vessel lumen was noted. It is suggested that a defect in the endothelium seen on scanning electron microscopy might constitute a capillary opening.

It has been claimed (Bloom and Fawcett, 1975) that the inner aspects of vein walls are better vascularized than that of arteries. In general, the media of veins is better vascularized than arterial media, and is provided with a more elaborate lymphatic supply. This may partly account for the fact that tumor invasion occurs far more frequently in veins than in arteries.

In addition to the SMCs, the principal structural elements of the vein wall consist of networks of collagen and elastin. While present throughout the vein wall, the adventitia is particularly rich in collagen. In both media and adventitia in human veins, collagen bundles appear to be arranged longitudinally, and occasionally spirally. A unique double criss-cross network of collagen fibers has been demonstrated in the thoracic IVC of dog, rabbit and cat (Franklin, 1931 and 1933), which is thought to explain the ability of these vessels to increase in length on inspiration by as much as 45 per cent. Spiral collagen fibers in human veins may subserve a similar role.

Like the SMCs, the elastic fibers are, in general, oriented longitudinally in the veins of the thorax and abdomen, and circularly in the limbs. These relationships help explain the ability of the veins in the extremities to serve as capacitance vessels and conduits, as well as the ability to resist compression of the centrally situated veins.

Venous innervation is provided exclusively by alpha adrenergic post-ganglionic fibers (Koelle, 1975). Beta receptors and cholinergic fibers have not been identified. Afferent neural elements, including Pacinian corpuscles, have been demonstrated.

Finally, the veins are unique in their possession of valves. The venous valves are essentially intimal folds arranged in the form of cusps whose free edges are oriented in the direction of blood flow. These cusps consist of little more than evaginations of endothelium resting on small amounts of connective tissue, particularly elastin, on the valve's luminal side. In the region of valve sinuses, the vein

wall is very thin, being approximately 20 to 25 per cent the thickness of the rest of the vein wall in the newborn (Kampmeier and Birch, 1927). The valves vary in number and location. They usually occur in pairs, but may be present singly. Most commonly, they are found at the mouth of, or immediately below, the entrance of major tributaries, and are increased in number as one progresses distally in the veins of the extremities (Ludbrook, 1966).

B. PHLEBOSCLEROSIS

Phlebosclerosis may be found bearing numerous appellations in the literature, including venofibrosis, fibrous endophlebitis, hyperplastic phlebitis and hypertrophy of the veins (Lev and Saphir, 1951). All these terms refer to a set of gross and microscopic alterations in vein walls similar in every way to its counterpart in the arterial system, arteriosclerosis.

Veins displaying this process are truly "hardened," with variable degrees of endothelial, SMC and connective tissue proliferation, leading to fibrosis, with or without calcification. Atherosclerosis, on the other hand, is a more severely necrotizing process, and is marked by a greater degree of SMC degeneration and disruption of elastic fibers, more extensive fibrosis and neovascularization and, of course, the presence of intra and extracellular deposits of lipid (World Health Organization, 1958). Indeed, "sclerosis of vascular walls is seen in its purest and simplest form in the veins, where degenerative changes remain minimal and where, owing to the scanty development of these lesions, we are most likely to gain an understanding of their origin" (Cramer, trans. by Geiringer, 1949).

As will be discussed, atheromas do occur in veins, but are extremely rare in situations other than those involving surgical interposition of veins as arterial conduits, and at the origin of the inferior vena cava. Phelbosclerosis is the characteristic response of the vein wall to venous hypertension.

A more rigid definition of phlebosclerosis (Lev and Saphir, 1951)

restricts the use of the term to degenerative changes in the vein wall of a relatively cell-poor character, with more extensive fibrosis and elastic fiber disruption. Endophlebohypertrophy is taken to represent an earlier stage in phlebosclerosis, marked mainly by subendothelial, as well as medial, accumulations of SMCs, collagen and elastin.

These changes appear to occur along a pathologic continuum, with venous changes of a degenerative nature being closely linked with abnormal rises in venous pressure. The distinction between endophlebohypertrophy and phlebosclerosis may also reflect the difference between physiologic adaptation and true pathology. It should be emphasized, however, that while sclerotic veins represent extremes of structural degeneration, they rarely appear in the literature as significant sources of morbidity and mortality. Venous varicosities in the lower extremity stand alone as common examples of phlebosclerosis associated with marked clinical symptomatology while thrombosis, as will be discussed in the section on venous bypass graft pathology, appears to actually constitute an early consequence of acute injury to veins.

Lev and Saphir introduce the term senile phlebosclerosis to refer to degenerative changes in vein walls associated with advancing age, but unrelated to underlying endophlebohypertrophy. Decreases in SMCs and elastic tissue as a function of age have been described in the great saphenous vein (Neumann, cited by Li, 1940) but such changes have not been confirmed in the portal vein.

While atherosclerosis may be generalized, phlebosclerosis tends to be segmental (Moschcowitz, 1960), usually appearing in areas where

the vein is exposed to mechanical or hydraulic stress. It has been argued (Hauswirth and Einsenberg, 1931) that the veins, by virtue of their greater delicacy and exposure to mechanical stress, should actually be more prone to sclerosis than arteries. In a study of 30 veins taken from patients under the age of 40, it was concluded that phlebosclerosis is a disseminated process. Franklin (1937) is of the opinion that phlebosclerosis is a condition primarily associated with arteriosclerosis and systemic hypertension. Later work has not borne out these positions.

Tedeschi (1941) observed gross adventitial thickening on the side of venous walls running parallel to arteries in the IVC, femoral and popliteal veins. While microscopic sections were not obtained, the side of the vein wall subjected to arterial pulsations appeared twice as thick as the opposite wall.

Gross thickening of vein walls in conditions unrelated to venous hypertension has been observed primarily in the Cramer-Schilling lesion, a plaque of subendothelial fibromuscular and collagenous deposits located at the junction of the left iliac vein and IVC and extending some two centimeters proximally into the cava (Geiringer, 1949) or at the confluence of the streams of the two iliac veins, along the posterior wall of the cava (Moschcowitz, 1960). This lesion has been noted in 50 per cent of post-mortem speciments, half of which contained fat, with or without calcifications (Schilling, as cited by Geiringer, 1949), and in all post-mortem specimens taken from patients over the age of 50 (Moschcowitz, 1960). Geiringer noted 108 such lesions in 245 consecutive necropsies (44%). Eleven of the

plaques were studied microscopically, and five consisted exclusively of intimal hyperplasia, with a normal or atrophic media, and hypertrophy of the adventitia. Five lesions stained positively for fat, although typical foam cells were not seen. No relationship was established between the presence of the Cramer-Schilling lesion and histories of venous congestion, hypercholesterolemia, and the presence of aortic atheromas.

Similar lesions were found in 160 of 208 (77%) IVCs (Stuart and Magarey, 1960). Only 40 of the patients studied had histories of underlying venous congestion. Sixty per cent of these lesions contained fat, while 10 per cent were grossly, and 16 per cent microscopically calcified. These features were not related to age or sex, but were never seen in newborns.

The confinement of these lesions to the origin of the IVC underlines the importance of purely mechanical factors in the pathogenesis of phlebosclerosis and venous atheromas. Both Geiringer, and Stuart and Magarey point out that the Cramer-Schilling lesion arises in a region where the right common iliac artery courses over the orifice of the left common iliac vein, compressing this vein as well as the origin of the cava against the lumbar spine. One instance of a sinistriposed IVC has been reported by Geiringer, with the Cramer-Schilling lesion located near the orifice of the right common iliac vein.

Proliferation of elastic, collagenous and SMC elements in the intima and intimal side of the media has been observed in 127 popliteal veins (Lev and Saphir, 1951) at the orifices of tributaries and in

regions adjacent to the popliteal artery. With advancing age, the lesions assumed an increasingly acellular character, with decreased numbers of SMCs and increased collagen in intima and media, as well as calcifications. Fat was not noted. This work suggests that sclerotic lesions in veins may arise even in the presence of minimal mechanical or hemodynamic stress.

Phlebosclerosis has been noted in numerous clinical conditions, the common denominator of each being the presence, over variable periods of time, of elevations in venous pressure. These conditions include the great veins, pulmonary veins and portal veins in congestive heart failure (CHF), and the portal, splenic and mesenteric veins in portal hypertension. Similar changes are present in venous varicosities. The more florid alterations seen in coronary artery bypass grafts form the subject of the next chapter.

Gross and Handler (1939) examined the SVCs of 21 patients who had had histories of chronic congestive failure of various etiologies. Macroscopic changes consisting of slightly raised yellow plaques were found in two of the 21 vessels, but histological sections revealed slight intimal thickening in four, and marked thickening in 14. Ten of the 21 veins showed thickening, splitting and reduplication of elastic fibers. The intimal changes consisted primarily of increased quantities of collagen, with six of the veins containing SMCs in the subendothelium. Medial hypertrophy, consisting of SMC proliferation and collagen deposition, were noted in 17 of the vessels. The presence of fat or calcifications is not described. These changes could not be correlated with the duration or severity of clinical symptomatology.

The authors describe the changes they observed as evidence of phlebosclerosis. Using Lev and Saphir's classification, the fact that elastic fiber fragmentation was found along with increased quantities of viable SMCs might place these changes somewhere between the extremes of endophlebohypertrophy and phlebosclerosis.

Similar findings were obtained in a study of 208 post-mortem cases, 30 of which involved histories of CHF, and an additional 10 of which were marked by histories of portal hypertension (Stuart and Magarey, 1960). Apart from the Cramer-Schilling lesion, no macroscopic plaques were seen in the 168 cases taken as controls. Of the 30 cases with CHF, 29 had lesions in the great veins, characterized by subendothelial accumulations of fibrous connective tissue, elastin and SMCs. Four of the lesions stained positively for fat. In addition, six of the 10 portal veins taken from patients with portal hypertension revealed similar changes. These histological alterations were found in regions displaying grossly visible, firm plaques, but even random sections showed thickening of the vein wall that was three to four times normal. In this study, duration and severity of venous congestion was seen to be related to the degree of observed pathology.

A comprehensive survey of phlebosclerosis has been performed at Mount Sinai Medical Center, New York City (Moschcowitz 1959, 1960, 1962, 1964; Moschcowitz and Strauss, 1964). This work suffers from containing a minimum of descriptive pathology, and the somewhat arbitrary position that any degree of intimal thickening is tantamount to phlebosclerosis. That phlebosclerosis might properly be regarded as an end-stage which follows from the ultimate inability of

proliferative responses on the part of the vein wall to cope with abnormal transmural pressures is not considered. The initial response and the final outcome are not clearly distinguished.

Nevertheless, Moschcowitz performed an important service by amassing a large body of data correlating structural changes in veins with clinical histories of venous congestion. In a pilot study of 100 consecutive portal veins, intimal thickening was never noted except in the presence of presumed venous hypertension (Moschcowitz, 1959). In 105 cases of CHF and intra and extrahepatic portal obstruction, intimal thickening was a constant finding, although exact figures are not provided. No lipid or reduplication of the internal elastic membrane was noted. In 162 cases of CHF, intimal collagenous thickening was found in 157 IVCs (94.6%), excluding the Cramer-Schilling lesion (Moschcowitz, 1960). In the 49 IVCs displaying the most marked changes, the duration of symptomatology was found to be double that of the other cases studied. In addition, 26 of the 49 cases (53%) had histories of valvular heart disease, suggesting relatively prolonged venous congestion. No relationship was established between the degree of intimal thickening and the presence of diabetes mellitus.

Intimal thickening was demonstrated in 151 pulmonary veins, of which 146 (97%) were taken from cases of CHF (Moschcowitz, 1962). There were concordance rates of 96% and 40% between pulmonary vein pathology and intimal thickening in the IVC and portal vein, respectively.

Further evidence for the cardinal importance of venous hypertension in the generation of adaptive and degenerative changes in the

vein wall may be gleaned from the fact that structural alterations were never found in the coronary veins from 50 patients with CHF (Moschcowitz, 1964). The free anastamoses between these veins and the Thebesian vein, coupled with the usual incompetence of the Thebesian valve in the coronary sinus, apparently prevent the development of undue increases in pressure.

The portal vein has been studied in 30 cases of cirrhosis of the liver (Wilson, 1961) and 26 cases of CHF, chronic airway disease and cirrhosis (Li, 1940). Controls taken from cases in which venous congestion was absent showed a complete lack of a subendothelial layer in two and 26 portal veins, respectively. Twenty nine of 31 portal veins studied by Wilson (one additional portal vein was obtained from a case or recurrent hematemesis and splenomegaly without cirrhosis) showed varying degrees of thickening affecting all layers of the vein wall, with SMCs and collagen accumulating in the subendothelium, and SMC hypertrophy in the media and adventitia. Three of the most advanced lesions displayed organizing thrombi within the intima, raising the possibility that organization and incorporation of thrombi may be involved in the most florid examples of hypertensive venous pathology. Li's findings are similar, with 20 of 26 cases (77%) showing proliferative lesions. Neither author found atheromas.

Sclerosis of the wall of the portal vein has been reported in a 21 year old female with Banti's Syndrome (Reich, 1942). All layers of the vein wall were affected, with SMC degeneration and replacement with hyalinized areas of connective tissue. Whiteley (1953) found a thrombosed portal vein in a 64 year old female with cirrhosis. An

area of marked fibrous intimal proliferation was seen proximal to the thrombus. This region was calcified, contained fibrin, and stained positively for fat. Risk factors for atherosclerosis are not noted.

An unfortunate drawback of this type of work is that correlations with duration and degree of clinical symptomatology and alterations in venous structure tend to be largely impressionistic. Data derived from portal manometry and cardiac catheterization would have been of interest in these studies, but the bulk of classical histological investigations involving sclerotic veins were performed before these tools came into general use. One semi-quantitative approach was taken by Wilson (1961), who established a positive correlation between structural changes in the portal vein in portal hypertension and spleen weight.

Venous varicosities are the most familiar examples of sclerosis in the venous system. The controversy regarding the primary or secondary role played by valvular incompetence in the generation of varicose veins has been reviewed by Ludbrook (1962).

Nicholson (1927) found thickening of the intima in venous varicosities, with SMCs accumulating in the subendothelium. Moving distally along affected veins, the amount of connective tissue was found to increase, accompanied by attenuation of elastic fibers. Stein, Rosenblum and Leather (1966) studied veins obtained from phlebectomies performed for varicosities. Intimal thickening was observed in the absence of subendothelial cellular proliferation. The intimal thickening consisted primarily of an increase in eosinophilic ground substance. Medial fibrosis was noted, and was accompanied by secondary elastosis.

The distribution of ATPase, succinic dehydrogenase, NAD diaphorase, alkaline phosphatase, acid phosphatase, glucose-6-phosphatase and non-specific esterase was found to be identical with that of normal veins. Atherosclerotic plaques, on the other hand, are marked by increased phosphatase activity within the intima. Fat was not identified. The histologic alterations which have been found in venous varicosities appear to constitute phlebosclerosis, strictly speaking. In addition, these findings suggest a possible pathogenetic role for stasis and anoxia, in addition to increases in pressure, in the development of these lesions.

The question of the temporal primacy of medial versus intimal thickening has been debated. Areas of medial hypertrophy with a normal intima have been found in the mesenteric veins (Li) and portal vein (Wilson) of patients with portal hypertension. Furthermore, Li reports regional differences in vein wall hypertrophy, with adventitial hypertrophy actually exceeding medial hypertrophy in the IVC in CHF and chronic airway disease. This selectivity, which was not confirmed by Wilson, is explained by the fact that the IVC is fixed at both ends, so that hypertrophy of longitudinal adventitial SMCs suffices to decrease wall tension in the face of increases in transmural pressure.

Partial ligation of the IVC of rabbits has been performed (Ts'ao and Spaet, 1967), with sections removed at fixed intervals for electron microscopy. The initial effect of increases in vein wall tension involved accumulation of mitochondria and lysosomes in endothelial cells, with dilatation of rough endoplasmic reticulum and proliferation of Golgi elements. This was seen within minutes. Autolysis of

endothelial cells was seen after one hour. After two hours, leukocytes and SMCs were observed in the subendothelial space. Medial changes were less dramatic, with SMC necrosis noted on the intimal side of the media.

More recent evidence derived from studies of the arterial system strongly suggests that myointimal thickening and medial hypertrophy unfold as an orchestrated process (Ross and Glomset, 1973; Fishman, Ryan and Karnovsky, 1975; Ross, Glomset and Harker, 1977). As Virchow stated in 1856, endothelial injury is the primary event in atherogenesis. Endothelial desquamation, with the concommitant release of platelet-borne mitogenic factors, appears to be of prime importance in the subendothelial accumulation of connective tissue and SMCs. In the absence of experimental evidence, one may speculate that a similar series of events occurs in injured veins. The role of the adventitia in this process is not clear, but adventitial SMCs may respond to similar factors, or they may be more dependant on the integrity of the vasa venorum.

How undifferentiated SMC precursors migrate through the internal elastic membrane is not clear. Evidence regarding the probable medial origin of intimal SMCs in veins is presented by Geiringer (1949), while Haust, More and Hovat (1960) provide strong electron microscopic evidence for the SMC characteristics of cells embedded in the connective tissue matrix of human atheromas.

The rapidity with which alterations of this nature develop may be gauged from reports of extreme intimal thickening in the portal vein of a 7 1/2 month old child with congenital biliary atresia

(Moschcowitz, 1959), and in the IVC of a 23 day old with multiple congenital cardiac anomalies (Moschcowitz and Strauss, 1964).

An additional issue raised by several studies involves the time sequence of elastic fiber deposition and degeneration in these lesions. In a series of 50 patients with left sided CHF, intimal thickening marked primarily by the presence of fibrous and elastic tissue (intimal fibroelastosis) was found in the pulmonary veins of 21, with a total of 46 displaying some degree of intimal thickening (Hutchins and Ostrow, 1976). According to Lev and Saphir, the progression of phlebosclerosis is marked by successive decrements in elastic tissue. On the other hand, Wilson (1961) found the greatest degree of elastic tissue deposition in the most fibrotic portal veins of patients with cirrhosis, particularly those with organized thrombi.

The opposite relationship has been found between the presence of organized thrombi and granulation tissue, and proliferation of elastic tissue in healed myocardial infarcts (Hutchins and Bannayan, 1971). Subendocardial fibroelastosis was the presumed response to increases in myocardial wall tension following infarction, except in regions containing organized thrombi. Moschcowitz (1960) denies that thickening of the internal elastic lamina or proliferation of elastic tissue occurs in sclerotic veins, while elastosis was a constant feature of venous varicosities examined by Stein, Rosenblum and Leather (1966).

Whether elastin is actually present in many of these lesions is an issue raised by Gillman et al. (1955), who studied "elastotically degenerated" arteries with a battery of fifteen histochemical stains.

Elastic fibers in these blood vessels were noted to be thicker and more granular than the elastin of the internal elastic membrane, and consistently failed to reproduce the membrane's staining properties. It remains to be established whether intimal and subendocardial fibroelastosis represents an adaptive increase in elastic tissue, a degenerative alteration of pre-existent elastin or, possibly, an entirely different material.

Of interest are alterations in vein walls which have not been directly linked to increases in venous pressure. Micronodular phlebosclerosis refers to focal accumulations of SMCs and connective tissue in vein walls which have been found to impinge on the lumens of 30% of renal and 51% of adrenal veins taken from 100 consecutive autopsies, averaging 62 years in age (Payan and Gilbert, 1968). Obliterative intimal fibrotic changes with medial sparing has been seen in primary pulmonary veno-occlusive disease (Carrington and Liebow, 1970; Heath, Scott and Lynch, 1971). The role of ionizing radiation, antineoplastic and immunosuppressive agents, arsphenamine, urethane and graft versus host disease in the pathogenesis of non-thrombotic proliferative lesions in the walls of centrilobular and sublobular hepatic veins has been reviewed by Berk et al. (1979).

It thus appears that increases in venous pressure are of central, although not exclusive, importance in the pathogenesis of structural changes in vein walls. A variety of initial injuries may set into motion a set of stereotyped responses leading to intimal thickening. Persistence of injurious stimuli, possibly in association with the affects of advancing age, leads to the development of sclerosis. The

time period over which these changes arise are telescoped into weeks and months in autogenous vein grafts.

C. AUTOGENOUS VEIN GRAFTS: ANATOMIC PATHOLOGY

The study of autogenous vein grafts (AVG) provides an opportunity for evaluating the contributions of a wide range of factors to the development of sclerotic and atheromatous changes in the vascular wall. Relative hypertension, stasis, turbulence, anoxia and hyperlipidemia have all been implicated in the pathogenesis of thrombosis, fibrous intimal proliferation (FIP) and atherosclerosis -- the principal pathologic alterations which have emerged from a large number of studies in experimental animals and man.

In 1906, Jose Goyanes of Spain performed what is thought to be the first AVG in man when he utilized a segment of popliteal vein to replace a resected syphillitic aneurism in the popliteal artery of a 41 year old man (Harrison, 1976). His achievement derived in part from the experimental work of Alexis Carrel who, with Morel at Lyons University and Guthrie at the University of Chicago, established the methodology of modern vascular surgery. In part for this work, Carrel received the 1912 Nobel Prize for Medicine or Physiology.

The development of radiologic contrast methods to localize arterial obstructions and the use of heparin to control the problem of intraoperative thrombosis has facilitated the application, over the last two decades, of AVGs in the treatment of atherosclerotic coronary vascular disease (ASCVD), peripheral vascular disease and renal artery stenosis.

Fifteen years ago, the first saphenous vein (SV) coronary artery bypass graft (CABG) was performed at the Methodist Hospital in Houston,

Texas. The case was not reported until 1973, five years after the technique for bypass grafting for ASCVD, culled from extensive experience at the Cleveland Clinic, was published by Rene Favaloro (1968).

It is beyond the scope of this discussion to review the large body of data which has accumulated since 1968 regarding the specific clinical indications for performing CABGs, and the correlations which have emerged between preoperative clinical and angiographic findings, and morbidity and mortality following medical or surgical therapy for ASCVD. Over 300,000 CABGs have been performed to date, of which over 1200 have been studied in a random, controlled manner. These controversial issues have been reviewed by McIntosh and Garcia (1978) and Hurst, King, Logue et al. (1978).

1. Morphologic Findings

Fibrous intimal proliferation has been a common finding in autogenous vein grafts. This entity appears to duplicate the time and pressure related lesions which have previously been identified *in situ* in the venous system. The rate at which the lesions develop, however, appears to be accelerated in the presence of systemic blood pressure and pulsatile flow.

A selection of investigations which have produced noteworthy examples of FIP in vein grafts is presented in Table 1. In general, early lesions tend to be relatively cellular, consisting primarily of SMCs in a loose matrix of collagen, elastic fibers (not a uniform finding), and acid mucopolysaccharides. With time, sclerosis supervenes and the intimal lesions assume a fibrotic, acellular character. Concommitant changes affect the media and adventitia. Interpretations of medial changes vary as to whether the media is spared in the short run, or actually displays some early degree of SMC hypertrophy before becoming fibrosed. Other controversial issues devolve around the rapidity with which FIP develops, the extent to which the process is progressive and stenosing, and, of course, the relative importance of individual pathogenic factors.

Alterations in venous grafts clearly reflect an inherent capacity of the intact vein wall to respond to a wide range of possible insults. An experiment involving the grafting of a formalin-fixed segment of heterologous IVC onto the common carotid artery of a dog (Klotz, Permar and Guthrie, 1923) as the clinical use of Dacron grafts in

TABLE 1: FIBROUS INTIMAL PROLIFERATION IN AUTOGENOUS VEIN GRAFTS

Author(s)	Type of AVG	Findings
Grondin et al., 1971a	CABG	Two 114 day old grafts with marked FIP
Marti et al., 1971	CABG	100% of grafts with FIP, (0 to 10 months), including two from intraoperative deaths (? pre-existent phlebosclerosis)
Vlodaver and Edwards, 1971	CABG	19 of 19 (100%) unchanged at up to 7 days, slight FIP in 2 of 2 (100%) at less than 30 days, marked FIP in 6 of 8 (75%) at 3.5 to 9 months
Hamaker et al., 1972	CABG	One graft with marked FIP at 15 weeks
Kern et al., 1972	CABG	Slight intimal edema and fibrosis in 7 of 7 at less than 30 days, marked FIP in 9 of 9 older than 30 days
Szilagyi et al., 1973	Femero-Popliteal SV graft	4 of 21 (19%) with FIP at average of 11 months
Barboriak et al., 1976	CABG	17 of 17 with FIP at 1 to 53.5 months, with 7 of 17 (41%) occluded entirely by FIP
Lawrie et al., 1976	CABG	17 of 17 with FIP at mean of 34 months
Bulkley and Hutchins, 1977	CABG	17 of 83 (21%) with FIP at less than 30 days, 14 of 14 with FIP at more than 30 days
Roscher and Kern, 1977	CABG	FIP seen in 3 of 26 post-mortem cases (0-21 months)

CABG - coronary artery bypass graft
FIP - fibrous intimal proliferation
SV - saphenous vein

peripheral vascular surgery (DeBakey et al., 1964) have shown that essentially inviable conduits may become fully endothelialized and, in the case of one Dacron graft, may accumulate lipid beneath the neoendothelium. A markedly thick subendothelial layer has not been demonstrated in such grafts. A characteristic feature of FIP is that the ratio of intimal to medial thickness often exceeds three, and even four (grade 3 and 4 intimal proliferation).

Some degree of intimal thickening may precede the transposition of SVs into the arterial system. Grade 1 and 2 intimal thickening was a uniform finding in 200 SVs taken from patients in the fifth and sixth decades of life (Lawrie et al., 1976). Florid FIP or atherosclerosis, however, has never been found in a SV prior to use as an AVG.

Saphenous veins are prepared for use as a bypass graft by mechanical dilatation, usually with heparinized saline. This is done to avert spasm in the graft. Considering the likely importance of endothelial injury in the etiology of intimal thickening, as well as atherosclerosis, the effects of dilatation on the venous endothelium is of interest.

Barboriak et al. (1976b) obtained three SVs which had been routinely prepared for coronary artery grafting, and found that one displayed focal areas of endothelial denudation. Both scanning and transmission electron microscopy were used to confirm these findings. One of two grafts removed after 50 months in place had an intact endothelium, with the other having areas of endothelial loss -- but with little adherent fibrin or platelets, suggesting that this finding was artifactual. Distension of canine cephalic veins at pressures

usually utilized in graft preparation (up to 600 mm Hg) has been seen to result in marked endothelial disruption, with increases in size, flattening and loss of microvilli in remaining endothelial cells, tearing of elastic fibers, partial detachment of the adventitia, and necrosis of medial SMCs (Ramos et al., 1976).

Focal loss of endothelial cells has been noted in two thirds of CABGs examined within hours of transplantation (Unni et al., 1974). Autologous cephalic and femoral veins used in CABGs in dogs were seen to have patchy areas of endothelial loss after only 24 hours in place (Brody, Angell and Kosek, 1972). In addition, canine SVs which had not been dilated, but simply placed in saline for 90 minutes, displayed greater than 50% loss of endothelium using en-face silver preparations. This loss progressed to 70% after 48 hours as end to end femoral artery grafts, with complete endothelial restoration apparent only after 12 weeks (Wyatt and Taylor, 1966).

Aside from endothelial loss, early CABG changes (those noted in grafts in place for less than seven days) have either been unappreciable (Vlodaver and Edwards, 1971) or, more frequently, have consisted of some degree of laminar fibrin deposition in the intima (Unni et al., 1974; Lie et al., 1977; Bulkley and Hutchins 1977 and 1978). Dewangee et al. (1978) demonstrated significant early platelet compartmentalization in SV CABGs in dogs. Indium-111 labelled platelets were injected intravenously after surgery, and gamma scintillation images showed activity in the grafts at eight and 32 hours post-operatively that was four to 15-fold increased with respect to the peripheral circulation and 25 to 100-fold increased with respect

to myocardium.

Early polymorphonuclear leukocytic (PMN) infiltration of the presumably injured graft wall has not been a uniform finding. Unni et al. (1974) note a modest degree of PMN infiltration in one CABG removed within hours of insertion, with more marked PMN infiltration and medial SMC necrosis seen in a second graft removed within 30 days of insertion. In general, PMN mediated injury has not been implicated as a major factor in CABG pathology. In experimental models, Stewart, Ritchie and Lynch (1974) implicate white blood cells in the propagation of mechanical injury to canine jugular and femoral veins, while Malone and Morris (1978) suggest that white cells are more than simply passive participants in the induction of thrombosis in rabbit ear veins injured by hypoxia.

Among the earliest changes which have been described in AVGs is a histochemical demonstration of increased ground substance (toluidine blue-positive material) and glucose-6-phosphate dehydrogenase activity in the intima of SV-femoral artery end-end grafts in dogs after 48 hours (McCabe et al., 1967). This increase in G6PD activity may be related to an early rise in RNA and protein synthesis in grafted veins.

SMC proliferation and acid mucopolysaccharide deposition may precede noteworthy deposition of collagen (Batayias, Barboriak et al., 1977). While one light microscopic study was able to detect FIP in CABGs less than 12 days old (Marti, Bouchardy and Cox, 1971), most workers have concluded that FIP is not evident before 30 days (Vlodaver and Edwards, 1971; Unni et al., 1974; Barboriak et al., 1978). Intimal accumulation of SMCs has been seen at 19 days in an electron microscopic

study of a CABG (Unni et al., 1974).

Increasing intimal fibrosis, marked by increased collagen deposition and SMC drop-out, appears in CABGs after about one year in place (Batayias, Barboriak et al., 1977). Many viable SMCs were still evident in a 21 month old densely fibrotic plaque (Kern, Dermer and Lindesmith, 1972).

The inner and outer SMC layers of the media may show fibrotic changes within days post-operatively, with some initial SMC hypertrophy occurring in the middle zone (Marti, Bouchardy and Cox, 1971). Older CABGs (weeks to months) have displayed medial SMC drop-out and increased fibrosis (Hamaker, O'Connel and Gomez, 1972; Kern, Dermer and Lindesmith, 1972). Similar findings have been noted in femeropopliteal SV grafts (Szilagyi et al., 1973). Batayias, Barboriak et al. (1977) found significant medial sclerosis only in CABGs one to three years old, with the media essentially unchanged before six months. Some workers (Vlodaver and Edwards, 1971; Unni et al., 1974) have been unable to find significant medial changes in CABGs three to nine months old. Considering the marked medial changes described in distended, pre-implantation grafts, some degree of structural restoration may occur in the media, as in the adventitia, before fibrosis ensues.

While the exact nature and time course of medial changes remains to be pinpointed, it appears that grafted veins do not assume the histologic characteristics of arteries ("arterialization"). In addition, the extensive necrotic changes observed in arterioles in malignant hypertension (Crawford, 1977) do not occur.

Structural alterations in the adventitia have, in general, paralleled those observed in the media, with proliferative and ultimately degenerative changes a frequent finding. Bulkley and Hutchins (1977) have commented on the contribution made by pericardial adhesions to adventitial thickening in CABGs, and Marti, Bouchardy and Cox (1971) have seen increased elastin deposition in the adventitia of such grafts. The disruption of the adventitia as a result of mechanical dilatation is severe, with complete restoration evident only after three months in experimental animals (Ramos et al., 1976).

The effects of transplantation into the arterial system on the vasa venorum vary. Mechanical dilatation, the care with which the surgeon preserves the adventitia (Ramos et al., 1976) and the solution used to dilate and temporarily store the veins (Abbot, Wieland and Austen, 1974) play important roles. Wyatt and co-workers (1964) found, using an India ink study, that two to six months are required for restoration of the architecture of the vasa in SVs used as femoral artery grafts in dogs.

CABG patency rates have been high. Patency has been demonstrated angiographically in 80 to 90% of CABGs studied within a month of surgery (Bourassa et al., 1972; Walker et al., 1972; Lawrie et al., 1976). Angipathologic correlations have been more difficult to establish. This is primarily because peri-operative myocardial infarction (Langou, Wiles and Cohen, 1978) and problems arising from or exacerbated by the surgical procedure itself, not graft closure, are the major causes of early death.

In the relatively few closed grafts studied following death

within the first 30 post-operative days, thrombosis has been a major finding. Occluding thrombi have been seen in 3 of 14 (21%) of grafts one week to one month old (Unni et al., 1974). Bulkley and Hutchins (1977) found that patients dying intraoperatively had CABGs free of occluding thrombi, the incidence rising to 10% (2 of 21) at up to three weeks, and 43% (6 of 14) at one to 54 months. Organized thrombi have been reported in grafts in place for 8, 28 and 30 days (Kern, Dermer and Lindesmith, 1972), 34 days (Marti, Bouchardy and Cox, 1971) and in two of eight grafts examined at three to nine months (Voldaver and Edwards, 1971).

Whether FIP progresses over time and becomes ultimately responsible for a significant proportion of graft failures is not entirely settled, but it appears that it does not. The angiographic correlate of FIP has been described as a diffuse, wavy narrowing of the graft wall (Szilagyi et al., 1973). Using angiographic data, Lawrie et al. (1976) contend that FIP becomes maximal at three months, and Barboriak et al. (1978) have found that intimal thickness in CABGs does not increase after six to nine months. Of 55 CABGs demonstrated to be patent at angiography one year after surgery, only three were occluded at three years (Lesperance et al., 1973). A non-critical decrease in caliber seen in 70% of grafts at one year did not progress thereafter. An additional indication of the tendency of FIP to become self-limited comes from evidence that progressive disability and death in patients with CABGs in place for longer than three to nine months is primarily due to progression of ASCVD in ungrafted vessels (Seides et al., 1978).

Several reports of graft closure entirely attributable to FIP suggest that the process is not a benign one, however. A few examples of this are listed in Table 1. The cases described displayed lumenal obliteration without superimposed thrombosis. An unresolved issue is the extent to which FIP affects a graft's predisposition towards thrombosis. Barboriak et al. (1976a) have found thrombi in older grafts displaying significant FIP. This has not occurred frequently, however, with the majority of thrombotic episodes appearing to take place in the immediate post-operative period.

Typical atherosclerosis has been seen with increasing frequency in CABGs as long term survivors of coronary revascularization procedures come to post-mortem examination. Atherosclerotic changes may develop with remarkable rapidity in autogenous vein grafts. For the most part, the observed changes conform with standard pathologic descriptions of atheromas. A summary of AVG atherosclerosis in both experimental and clinical settings, along with the presence or absence of appropriate risk factors for ASCVD, is presented in Table 2.

TABLE 2: ATHEROSCLEROSIS IN AUTOGENOUS VEIN GRAFTS

Experimental

Author(s)	Model	Findings
Sako and Varco, 1962	Dog VC-AA graft (10 years)	Extensive fibrosis (+) sudanophilic material in intima, (-) in adjacent aorta
Friedman, 1963	Rabbits JV-AA grafts (8 to 12 wks). Some thrombosed with Mg-Al spiral. (+) AS diet	Grafts in hyper-C rabbits: Typical AS Thrombosed grafts: AS like that in intact aorta after experimental thrombosis. Non-thrombosed grafts (controls): no AS
Penn et al., 1965	15 dogs JV or FV to CA or FA. Thyroidectomy + AS diet. Avg. 87 days Avg. C 443	(+) FIP, lipid-laden SMCs in intima, (-) extracellular lipid
Wyatt and Gonzalez,	Dogs JV-CA grafts (9 days-3 weeks) Thyroidectomy, AS diet Avg. C 300-900	Sudanophilic material. foam cells even in normo-C dogs; avg. C less than 301: 80% patent, greater than 899: none patent
King and Royle, 1972	Rabbits FV-FA grafts (10 days-4 months) AS diet	100% AS Atheroma mainly on suture lines. More severe AS in rabbits put on AS diet before surgery vs. those on diet after surgery
Haimovici and Maier, 1974	JV-AA (avg. 11 months)	(+) AS
	JV-CA, JV-FA (avg. 26 months) End-end and end-side grafts on opposite sides. Thyroidectomy, AS diet to avg. C of 1000	Minimal AS in end-side grafts (o) AS in end-end grafts - these were primarily thrombosed

TABLE 2 (Cont'd)

Author(s)	Model	Findings
McCann et al., 1975	Rhesus monkeys JV-IIA grafts (6 months) AS diet. Avg. C 648, avg. TG 189	(+) FIP in all grafts, Hyper-C monkeys: lipid laden intimal SMCs seen on electron microscopy

Clinical

Author(s)	Type of AVG/Risk Factors	Findings
Ejrup, Hiertonn and Moberg, 1961	FV-FA graft 41 months Avg. C 283	Marked AS in distal graft with proximal sparing (+) superimposed thrombosis in AS plaque
Beebe, Clarke and DeWeese, 1970	Fem-pop SV graft (57 months) Avg. C 735	Intimal and medial fibrosis, (+) intimal lipid laden macrophages, cholesterol clefts
Allard, Russito and Goulet, 1972	CABG Angiographic Study	
	TG 287	Graft closure at 2 weeks
	TG 244	Severe stenosis at 1 year
	TG 224	Wide patency at 1 year (Serum C not predictive)
Barboriak, Pintar and Korns, 1974	CABG	
	6-61 months	6 of 8 (+) AS
	h/o DM	6 of 6 (+) AS
	Hyper C or TG	6 of 6 (+) AS
Stehbens and Karmody, 1975	Arteriovenous SV fistula for hemodialysis 12 months, C 200-290 (+) mild HTN	(+) AS Lipid laden SMCs in loose intimal connective tissue matrix

TABLE 2 (Cont'd)

Author(s)	Type of AVG/Risk Factors	Findings
Farry, Hammond, Cohen and Wolfson, 1976	CABG 4 years C 202 TG 316 (+) cigarette smoking	(+) AS - clefts of necrotic material surrounding cholesterol ester crystals (+) foam cells, calcification, hemosiderin laden macrophages
Barboriak et al., 1977	CABG 59 months C 295 TG 252	(+) AS Large intra-intimal necrotic mass, lipid laden macrophages
	CABG 20 months Type IV Hyper-lipoproteinemia	(+) AS with lipid laden macrophages
Batayias et al., 1977	CABG older than 3 years	3 of 6 (+) typical AS
Lie, Lawrie and Morris, 1977	CABG (-) DM, HTN	
	59 grafts One year	(-) AS regardless of lipid profile
	40 grafts 13-75 months	(+) AS in 11 of 14 (79%) from hyperlipidemic patients, and 3 of 26 (11%) from normolipidemic patients
Bulkley and Hutchins, 1977 and 1978	CABG Older than 30 days	50% with lipid laden macrophages without other changes suggestive of AS. Earliest such change noted

TABLE 2 (Cont'd)

Author(s)	Type of AVG/Risk Factors	Findings
Barboriak et al., 1978	CABG 1-6.5 years	50% of grafts older than 3 years (+) typical AS
Pintar et al., 1978	CABG 5 years 62 yo male, (+) h/o HTN, family h/o ASCVD, C & TG nl	Both grafts displayed typical atherosclerotic aneurysms
	CABG 6 years 46 yo male C 228 TG 322-390 Type IV pattern	

C - serum cholesterol in milligrams per deciliter,
TG - serum triglycerides in milligrams per deciliter
AA - abdominal aorta CA - carotid artery FA, FV - femoral artery, vein IIA - internal iliac artery JV - jugular vein VC - vena cava
DM - diabetes mellitus HTN - hypertension h/o - history of

2. Etiology

A great deal of evidence has been adduced implicating endothelial injury as a key trigger in the set of platelet-SMC interactions which lead to arterial intimal thickening (see references cited on page 19). The precise biochemical nature of this process, the roles played by membrane interactions between cellular elements and the various components of the ground substance, and the manner in which the accumulation of lipid in the vascular wall is mediated, are subjects of active research.

The deleterious effects of surgical transplantation may be so severe as to induce FIP in arteries simply resected and reanastamosed into their sites of origin (McCann et al., 1979).

An evaluation of the possible contributing factors towards thrombosis in AVGs presents interesting problems. The distinction between "red" erythrocyte-fibrin thrombi and "white" platelet-fibrin thrombi has been reviewed by Mustard et al. (1962). The former usually arises in the setting of stasis, a condition most frequently extant in the veins, while the latter appears to arise primarily as a result of hemodynamic stress and injury in the arterial system. Relative stasis often coincides with hemodynamic stress in AVGs.

Most of the thrombi which have been described in CABGs appear organized and, occasionally, recanalized, making it difficult to invoke particular pathogenic mechanisms purely on morphologic grounds. Barboriak et al. (1978) demonstrate a fresh thrombus in a CABG which consisted primarily of crenated erythrocytes in a fibrin net. Platelet-fibrin thrombi have been noted in CABGs inserted in dogs (Brody,

Angell and Kosek, 1974). The work of DeWangee et al. (1978) suggesting early platelet compartmentalization in canine CABGs has been described above (page 28).

A more fruitful approach may involve an appreciation of the localization of both thrombi and FIP in vein grafts. The frequent presence of arterial intimal thickening at branch points, the proximal regions of branches, and at bifurcations, is well known. At such points, changes in laminar flow patterns (turbulence) and unusually severe shear and drag forces tend to prevail (Gessner, 1973; Glagov, 1973). Such stresses may adversely affect the vessel wall not only on the basis of direct endothelial injury but, along with increases in wall tension secondary to elevations in blood pressure, may contribute towards changes in endothelial permeability. As a result, "insudation" of plasma components may occur (Walton, 1975), particularly LDL and fibrinogen.

Paradoxically, regions of low shear force in the thickened boundary layers prevailing in the outer wall of a branch may lead to wall thickening in arteries by permitting, as a result of relative stasis in that boundary layer, increased passage of plasma components into vessel walls (Caro, Fitz-Gerald and Schrotter, 1969). Restriction of thrombosis, FIP and AS to particular regions of specific graft segments has not been remarked on. Enhanced venous susceptibility to fluid mechanical disturbances may prevent the sparing of one side of a vein wall with respect to the other.

Pathologic changes may occur with greater frequency at particular regions along the length of a graft. Of seven thrombi noted by Lie,

Lawrie and Morris (1977) in CABGs, four were present in the distal and one near the proximal anastamosis. Similarly, Bulkley and Hutchins (1978) found that obstructive changes in CABGs are almost invariably confined to the juncture of graft and coronary artery.

Several observers have disagreed as to whether FIP is a segmental process. In femoral vein-iliac artery grafts in dogs, Bond et al. (1976) did find that FIP predominated near the proximal and distal graft anastamoses, and in canine jugular vein-iliac artery grafts, FIP was found mostly at the suture sites (Faulkner et al., 1975). Other workers have claimed that FIP is a circumferential process affecting the length of the vein wall (Roscher and Kern, 1977). One cannot rule out that the hemodynamic stress imposed by exposure to arterial pressure should affect, to some degree, the entire length of a patent graft. FIP may arise initially at branch points and sites of suture-induced trauma before extending to the rest of the graft. Many more grafts will have to be examined before the evolution of FIP can be securely delineated. With the data now available, it appears that thrombi do arise primarily at or near anastamotic sites.

A particular pattern of atheroma distribution in CABGs has yet to emerge. Exceptions include the findings of Ejrup, Hiertonn and Moberg (1961) and King and Royle (1972) (see Table 2).

An "ideal" angle for CABG placement into the aorta has been calculated by Kennedy et al. (1974). By matching coronary artery impedance to the aortic pressure wave over a cardiac cycle in a computer model, ninety degrees was found to be the angle most likely

to prevent the development of significant shear forces within the graft. Some support for this finding has been seen in peripheral vein grafts in dogs (Bond et al., 1976), with grafts taking off at acute angles developing significantly more FIP than those taking off at 90 degrees. Grafts taking off at 120 degrees also showed some apring, however. No effect of angle variation at the aortic anastamosis was noted by Breyer et al. (1976) in 13 SV CABGs in dogs. A survey of CABG-aortic angles and CABG pathology in post-mortem cases has yet to be performed. Once again, however, consideration should be given to the likelihood that changes attendant on prolonged graft patency might obscure any initial changes which might be observed.

Factors expected to enhance stasis and, consequently, the contact time of circulating molecules (particularly LDL and fibrinogen) and formed elements with the vein wall, have been gauged by means of intra-operative measurements of graft flow rate and post-operative angiographic determinations of distal grafted vessel runoff and stenosis.

Angiography performed 10 to 21 days post-operatively on 70 patients (103 grafts) who underwent coronary revascularization showed that all grafts with intra-operative flow rates less than 20 milliliters per minute, or which failed to respond to papaverine, were closed, while all those with flow rates greater than 45 ml/min, or which responded to papaverine, were open (Grondin et al., 1971b). Failure to increase flow on administration of papaverine suggests distal coronary artery stenosis. Fifty per cent of CABGs with intra-operative flow rates less than 20 ml/min have been seen to be completely obstructed at three

months in another study (Walker et al., 1972), with 90% patency achieved with flow rates greater than 41 ml/min.

In a review of post-operative angiograms performed on 276 femeropopliteal SV grafts at Columbia-Presbyterian Medical Center, 57% of grafts having three vessel runoff were patent at five years, while all grafts with zero vessel runoff were closed (Buda et al., 1976). Koontz and Stansel (1972) examined similar data accumulated at Yale-New Haven Hospital and concluded that outflow tract runoff was not as critical in maintaining graft patency as the diameter of the grafted vein, with veins less than five millimeters in diameter especially prone to closure. End-arterectomy was also implicated as a risk factor, suggesting that subsequent arterial obstruction could contribute to graft closure by promoting stasis.

CABG patency has been related to the size of the grafted artery, with grafts placed on coronary arteries less than 1.5 millimeters in diameter practically assuring graft closure (Bourassa et al., 1972; Lesperance et al., 1972). A correlation was also established in these studies between poor distal vessel runoff and graft closure. The different emphases which have been placed on the sizes of graft and grafted vessel suggests that an evaluation of the effects of the ratio of the two diameters might provide additional information.

Furuse et al. (1972) showed that halving of the ratio of graft to grafted vessel diameter leads to nearly a doubling of flow rate in experimental CABGs in dogs. A decrement, however, was observed in total flow.

Laplace's law (tension = pressure x radius) predicts that an increase in graft diameter might result in elevated graft wall tension. In addition, increases in graft diameter are likely to be directly related to increases in intra-graft turbulence, since the Reynolds number is a direct function of vessel diameter. Since the Reynolds number is also a direct function of flow rate, consideration should be given to the possible deliterious effects of high, as well as low, flow rates.

Grafts in which the flow rates have been highest -- SV aorto-renal bypass grafts -- have not been immune to FIP. Flow rates in such grafts exceed those in CABGs by factors of three to four (Stanley et al., 1973; Dean et al., 1974). Faulkner et al. (1975) achieved an average 6.7 fold increase in flow rate in canine peripheral vein grafts by creating distal arteriovenous fistulas. FIP was more marked in the high flow grafts as compared to controls, but less than that developing in grafts in which stasis had been developed by means of distal arterial stenoses. It is possible that high flow rates may result not only in enhanced turbulence, but in a "suction" effect which might lead to endothelial damage (Texon, Imparato and Lord, 1960).

Increases in graft diameter following placement into the arterial system are known to occur commonly. Minimalization of post-operative graft distension in experimental animals has been achieved by encasing grafts in loose mesh prostheses. The effect of this intervention has been to markedly decrease the extent of observed FIP in jugular vein-common carotid grafts in dogs (Hostetler et al., 1976; Karayannacos

et al., 1978) versus untreated controls. Whether the prevention of graft distension or pulsatile flow is of greater import cannot be answered by these studies.

Graft/artery disproportion has been implicated in the etiology of graft closure purely on technical grounds. Actual incorporation of part of the grafted coronary artery into the anastamosis has been claimed to be the principal cause of early graft failure (Bulkley and Hutchins, 1977). Large graft/artery diameter ratios (exceeding two) was demonstrated to lead to kinking at the anastamotic site by means of epoxy resin cast injections of distal CABG anastamoses in isolated swine hearts (Young et al., 1978).

Technical error in angiographically pinpointing sites of coronary artery obstruction, or intraoperative error, was felt to account for the observation that 44% of CABGs studied in one series were grafted onto coronary arteries which were greater than 75% narrowed within two centimeters distal to the anastamosis (Spray and Roberts, 1977).

Anoxia constitutes an additional contributing factor towards vein graft pathology. Disruptive changes in the adventitia and vasa venorum have been described above. While mechanical dilatation or adventitial stripping of veins used as femoral artery bypass grafts in dogs has been found not to increase the degree of observed FIP (Storm et al., 1975), other investigators have come to opposite conclusions.

Canine cephalic and femoral veins used as CABGs have displayed fibrotic changes similar to those seen in control veins left in place,

but devascularized (Brody, Angell and Kosek, 1972). Autogenous femoral veins used as femoral artery bypass grafts in dogs developed FIP regardless of whether the vasa were interrupted, but those grafts with intact vasa displayed minimal medial pathology (Brody, Kosek and Angell, 1972).

Additional work assessing the role of venous vascularization in the pathogenesis of FIP has involved the encasement of grafts in non-porous prostheses. FIP has been noted to be especially severe in this setting, with the extent of intimal fibrosis correlating inversely with the degree of graft revascularization (Hostetler et al., 1976; Karayannacos et al., 1978).

The extent to which interruption of the vasa venorum contributes to the development of atheromatous changes in vein grafts is unclear. Dixon (1961) was of the opinion that intact vasa were required to prevent intimal anoxia in arteries, and resultant decreases in protein synthesis and micellar dispersion of fat. On the other hand, it has been claimed that transinitimal passage of oxygen and substrates from the vessel lumen is responsible for the nourishment of the arterial endothelium (Glagov, 1973). Whether this is true of the veins, or whether their increased degree of vascularization is due to an inability of the sluggish venous circulation to provide for all the metabolic needs of the venous intima, is still not settled. Interestingly, the development of atherosclerosis in rabbit arteries has been shown to be accompanied by proliferation of intimal arterial vasa which are highly permeable and which appear to deposit lipid which becomes engulfed by proliferating, actively phagocytizing intimal cells

(Friedman et al., 1962). Long term encasement of venous grafts in non-porous prostheses in experimental animals placed on an atherogenic regimen might aid in properly weighing the influences exerted by devascularization and revascularization in the development of venous atheromas.

An intriguing question is the extent to which veins and arteries differ in their susceptibility to atherosclerosis. That veins in their native positions are usually resistant to such changes, except in the case of the Cramer-Schilling lesion, has been reviewed above.

Haimovici and Maier (1971) transposed segments of aorta into jugular veins in dogs, and placed the animals on an atherogenic regimen. Remarkably enough, the aortic grafts developed atheromatous changes similar to those found in the native aorta, suggesting that the susceptibility of arterial tissue to atherosclerosis is of sufficient magnitude to manifest itself even in a low pressure system. Aortic implants into the IVC of rabbits, however, failed to develop atherosclerotic lesions comparable to those seen in AVCs, except when the aortic grafts had been previously damaged by means of a Magnesium-Aluminum thrombogenic spiral (Friedman, 1963).

Filtration of serum under positive pressure through excised iliac arteries and veins (Wilens and McCluskey, 1952) demonstrated that both vessels are highly permeable to low molecular weight compounds, but the filtration rate is considerably higher in the veins. Retention of serum lipids, however, was higher in the arteries. It remains to be seen whether the structural alterations which occur in AVGs permit greater entrapment of "insuded" lipid over time. While even the most

severely fibrosed venous varicosities have been found to be free of fat, fibrosis in the face of much higher pressures and pulsatile flow may provide conditions which do permit such changes to occur.

Exposure to systemic pressure may alter lipid metabolism in vein grafts. Using carbon-14 labelled acetate to follow the synthesis of cholesterol and triglycerides, Larson, Hagen and Fuchs (1974) found that explanted canine AVGs incorporated far more counts into all lipid classes than control arteries, but only slightly more than control veins. While one must be cautious in coming to conclusions on the basis of tissue culture experiments, it might be that the inherent capacity of the veins for atherogenesis is greater than had been realized, particularly under the conditions extant in venous arterial conduits.

Rossiter and co-workers at Stanford University (1974) compared the pathologic changes in canine SV and internal mammary artery CABGs. Some dogs were placed on atherogenic regimens. Among the dogs on regular diets, all SV grafts displayed FIP after three to 15 months, with internal mammary artery grafts displaying no such changes. Of grafts placed in dogs made hypercholesterolemic, 10 of 12 SV grafts and 2 of 6 IMA grafts developed diffuse atherosclerosis. This further suggests that veins may be particularly susceptible to the combined effects of relative hypertension and hyperlipidemia, with arterial injury arising only when hyperlipidemia is combined with the transmural pressure already extant <u>in situ</u>.

It is possible that the initial suprastructure for the atherosclerotic plaque in both veins and arteries consists of platelet-fibrin

mural thrombi which, when organized over time, assume the morphologic characteristics of true atheromas. Evidence for this possibility has taken a great deal of time to develop, ever since Von Rokitansky proposed this hypothesis in 1852.

A beautiful series of photomicrographs published by Duguid (1946, 1949) of serial sections taken through atherosclerotic coronary arteries and aortas revealed the almost imperceptible manner in which typical atherosclerotic plaques blended into regions containing organized thrombi. Immunofluorescent localization of fibrin deep within atheromas has been reported by Woolfe (1961). More superficial and diffuse fibrin deposits in early atherosclerotic plaques, on the other hand, may be due to post-mortem insudation of fibrin (Florey, 1960).

In a similar manner, it may be that FIP is the morphologic equivalent of organized deposits of fibrin (Bulkley and Hutchins, 1977 and 1978; Lie et al., 1977). Such a mechanism for intimal thickening in vein grafts has been distinguished from the reactive intimal fibroelastosis seen in the intima of veins subjected to the less elevated pressures caused by left sided CHF, as has been previously discussed.

Clinical risk factors for the development of vein graft atherosclerosis have not been established, with the exception of hyperlipidemia and,particularly, hypertriglyceridemia in CABGs (see Table 2). Only anecdotal reports are extant linking AVG atherosclerosis with cigarette smoking, hypertension and diabetes mellitus. Further clarification of the contributions made by risk factors important in the etiology of ASCVD to vein graft atherosclerosis should be forthcoming. Patients with CABGs are not likely to be afflicted with

untreated hypertension, however. In addition, the relative increase in blood pressure between normo and hypertensive patients is obviously minimal compared to the magnitude of the pressure rise involved in transplantation from the native venous system.

What emerges from the pathologic study of AVGs is that simple, all-encompassing explanations are as inadequate in understanding the observed findings as they are in connection with arterial disease. Response to anoxic or mechanical injury, transudation from the circulation, organization of thrombi are not mutually exclusive events. They all may be valid ways of approaching the structural changes which have been observed in the venous wall. Each may initiate or temper the development of venous pathology. Further study of AVGs should help clarify the extent to which FIP is a necessary precursor of the atheromatous plaque, and the importance of individual pathogenic factors in the development of each lesion.

TABLE 3: FACTORS CONTRIBUTING TOWARDS INTIMAL THICKENING IN AVGs

---

I. Stasis

1. Increased graft diameter
2. Distal or proximal arterial obstruction
3. Poor distal arterial runoff
4. Surgically induced narrowing at anastamotic sites

II. Direct Endothelial Injury

1. Increased wall tension in presence of arterial pressure
2. Mechanical dilatation
3. Interruption of the vasa venorum
4. Effects of suturing
5. Effects of medium used to preserve veins before implant
6. ? effects of white blood cells

III. Turbulence and Shear/Drag Forces

1. Angle of graft placement
2. Narrowing at anastamotic sites
3. Increased velocity of graft flow (decreased graft diameter)

IV. Hyperlipidemia

Direct stimulus for SMC proliferation

V. Thrombosis

1. Secondary effect of direct endothelial injury and stasis which may increase stasis and turbulence, and act as direct stimulus for SMC proliferation
2. ? ultimate organization as morphologic equivalent of fibrous plaques

VI. Transudation

? changes in endothelial permeability and their significance

SECTION II: IMMUNOFLUORESCENT CHARACTERIZATION OF COLLAGEN TYPES I AND III IN NORMAL SAPHENOUS VEINS AND AORTOCORONARY SAPHENOUS VEIN GRAFTS

Collagen represents one of the largest components, by weight, of most connective tissues, particularly that of skin, bone, blood vessels, tendon and cartilage. Twenty per cent of the dry weight of lung may be attributable to collagen, and up to 70% of that of human skin. This protein has multiple functions, including the provision of tensile strength to tissues, as well as shape, flexibility and form; and the establishment of anatomic, and possibly functional, boundaries among cells and tissues (Gross, 1974).

The biosynthesis, structure and metabolism of collagen have been intensively studied. Excellent brief (Miller and Matukas, 1974; Uitto and Lichtenstein, 1976) and comprehensive (Bornstein, 1974) surveys of collagen biosynthesis have been published. Collagen degradative pathways have been reviewed by Perez-Tamayo (1978).

The collagen molecule is composed of a triple helical arrangement of three polypeptide chains, each approximately containing 1000 amino acids (95,000 daltons). The coiled triple helical structure has an axial distance of 2.9 Å between amino acid residues, rather than the 1.5 Å seen in the alpha helix. The dimensions of the molecule are about 15 Å by 3000 Å.

When organized as fibers, the arrangement of the collagen molecules has been seen to vary under electron microscopy with the method of preparation of the examined materials. "Native" fibers appear to

have repeating axial periods of 640 Å, or one quarter the length of a collagen molecule. These fibers consist of collagen molecules arranged in parallel register, but staggered by one quarter of their lengths. Different periodicities, representing variations in the dispositions of individual collagen molecules in whole fibers, are seen when collagen is extracted with citrate buffer and dialysed against water (Fibrous Long Spacing - FLS - arrangement), or treated with Adenosine Triphosphate in acid solution (Segment Long Spacing - SLS - arrangement).

The synthesis of the collagen triple helix involves a concerted series of intra and extracellular steps, with post-translational modification of individual amino acid residues playing prominent roles in the generation of the final structure.

Repeating amino acid residue triads of the general form X-Y-gly are characteristic of the collagen molecule, and appear essential to the maintenance of the molecule's stability. The first residue of this triad is usually proline, followed by either hydroxyproline or hydroxylysine.

The initial product of ribosomal translation of a collagen chain messenger RNA is a molecule of higher molecular weight than that of the native collagen molecule. These precursor chains are usually 200 to 300 amino acids longer than the final product at the carboxy terminus, and about 100 amino acids longer at the amino terminus. These extension peptides tend to be richer in acidic amino acids, and poorer in glycine, proline, and hydroxyproline than the rest of the collagen molecule.

Hydroxylation of proline and lysine residues begins while the precursor, or procollagen, chains are being assembled on ribosomes. Lysine and proline hydroxylases, specific for lysine and proline residues in the Y position, operate in conjunction with Iron (II), alpha ketoglutarate and ascorbic acid as cofactors.

An additional non-mRNA template directed synthetic step involves glycosylation of newly hydroxylated lysine residues. An O-glycosidic linkage is formed between the 3-hydroxy group of hydroxylysine, and galactose. Glucose may then be added to the galactosyl moiety. Specific galactosyl and glucosyl transferases are utilized in this step, with Uridine Diphosphate-Sugar as the carbohydrate source, and Manganese (II) as cofactor.

The three precursor chains associate into a triple helix, a process most likely facilitated by relatively specific interactions between the terminal extension peptides. These interactions are not purely non-covalent. The rate of formation of interchain disulfide bonds between half-cysteine residues in the extension peptides is proportional to the rate of formation of the triple helical product, procollagen.

Procollagen is secreted into the extracellular space, where endopeptidases capable of removing the amino and carboxy terminus extension peptides operate. These procollagen peptidases permit the spontaneous formation of insoluble collagen molecules and fibers from the soluble procollagen molecule.

Additional stability and tensile strength is afforded the newly formed collagen molecule by specific cross-links. The epsilon amino

groups of some lysine and hydroxylysine residues are first converted into aldehyde groups. This oxidative deamination is mediated by lysyl oxidase, an enzyme which is Copper (II) dependent. These terminal aldehyde groups may then form stable Schiff bases with the unaltered epsilon amino groups of other lysine or hydroxylysine residues, a cross-link most often associated with interchain interactions; or two aldehyde groups may form a bond through an aldol condensation, a common form of intramolecular linkage. The steps involved in collagen biosynthesis are schematized in Figure 1.

Collagen degradation is effected by cleavage of a single, specific peptide bond along the collagen helix recognized by specific collagenases. Collagenases have been isolated in numerous species, including crabs and flatworms, and in a large number of human connective tissues, as well as human platelets, granulocytes and macrophages.

Mechanisms controlling collagen synthesis and degradation are not well understood. Collagen biosynthesis has been associated with formation of polyribosomal aggregates, the activity of prolyl hydroxylase, the size of the intracellular proline pool, and the possible negative feedback effects of free terminal extension peptides. Collagen degradation may be related to the degree of cross-linking in each molecule, with highly cross-linked molecules being particularly stable. The nature of the molecular interactions involved in the stimulation and repression of collagen synthesis and breakdown remain unknown.

Increasing attention is being directed towards the elucidation of different collagen types. The structural and functional individuality of each collagen molecule is conferred by the association of

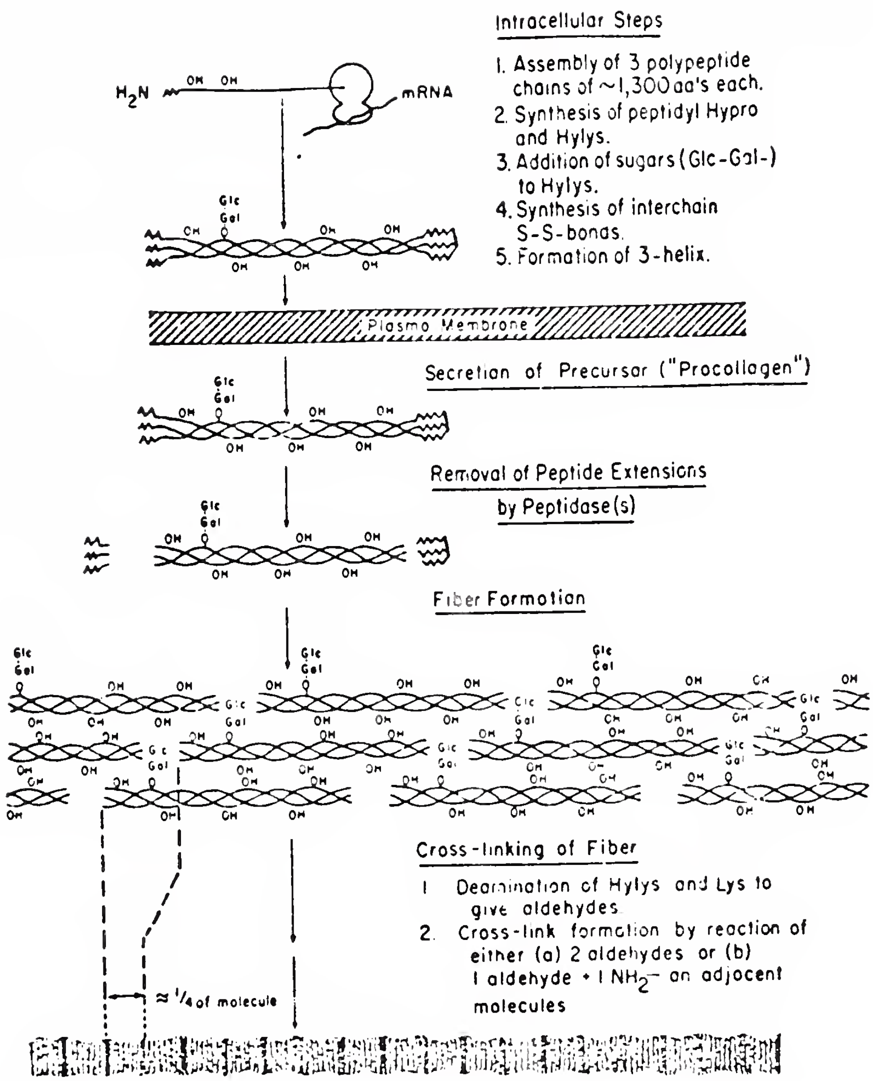


Figure 1. Schematic representation of collagen biosynthesis. From Uitto and Lichtenstein (1976).

distinct collagen polypeptide chains. The nomenclature and localization of collagen type I, II, III, and IV is summarized in Table 4. Each polypeptide chain is synthesized in precursor form, and is called a pro-αchain.

TABLE 4: HUMAN COLLAGEN TYPES

Type	Chain composition	Chain	Relative amino acid compositions: 4-Hyp	3-Hyp	Hyl	Gly	½ Cys	Predominant tissue distribution
I	$[\alpha 1(I)]_2\alpha 2$	$\alpha 1(I)$	93	0.8	4.9	346	0	Tendon, bone, dermis, ligament, lung, heart valve, fascia, scar, cornea, liver, dentin, cardiovascular tissue
		$\alpha 2$	83	0.8	9.2	347	0	
II	$[\alpha 1(II)]_3$	$\alpha 1(II)$	97	2	14	326	0	Cartilage, nucleus pulposus, notochord
III	$[\alpha 1(III)]_3$	$\alpha 1(III)$	124	0	5	354	2	Cardiovascular tissue, lung, liver, dermis, intestine, leiomyoma
IV	$[\alpha 1(IV)]_3$	$\alpha 1(IV)$	130	11	45	310	8	Basement membranes

Amino acid composition is expressed in residues/1000 amino acid residues. Values for type I and III collagen are from human dermis, type II is from human articular cartilage, and type IV from human glomerular basement membrane. Modified from Pinnell (1978).

The structure of type I, II and III collagen is relatively well understood. Methods of isolation, purification and characterization of these collagens may be found in the references cited in Table 5. The structure of type IV collagen is more controversial, and may actually be similar to that of procollagen (Orkin et al., 1977).

TABLE 5: ISOLATION AND CHARACTERIZATION OF TYPE I, II AND III COLLAGEN - SOURCES

Type I Collagen	Type II Collagen
Piez, Eigner and Lewis (1963)	Miller and Matukas (1969)
Rauterberg and Kuhn (1971)	Miller and Lunde (1973)

Type III Collagen

Chung and Miller (1974)

A fifth collagen type has been isolated from human placentas. This protein appears to be composed of two distinct collage polypeptide chains, call αA and αB (Burgeson et al., 1976). The stoichiometry of the type V collagen triple helix has not been conclusively demonstrated. A recent indirect immunofluorescent study showed that type V collagen is found throughout normal human lung, but primarily in association with basement membranes (Madri and Furthmayr, 1979a). Antibodies raised to this collagen were shown not to cross react with the previously demonstrated basement membrane collagen, type IV.

An additional collagenous protein has been isolated from Rhesus monkey SMC cultures (Mayne, Vail and Miller, 1978). Called CP45, it has a molecular weight of 45,000 daltons, and its structure and function remain to be determined.

Not surprisingly, several disease states have been linked to specific defects in collagen biosynthesis. These disorders, ranging from acquired deficiency states to inborn errors of metabolism, have been reviewed by Uitto and Lichtenstein (1976) and Pinnell (1978).

In the work described below, indirect immunofluorescent microscopy using specific anti-type I and anti-type III antibodies were used to evaluate the presence of these collagens in saphenous vein aortocoronary bypass grafts and ungrafted saphenous veins. As will be discussed, alterations in collagen type, in addition to absolute increments or deficits in collagen biosynthesis and degradation, may be of pathological significance.

MATERIAL AND METHODS

1. Primary and Secondary Antibodies

Antibodies to type I and type III collagen were provided by Dr. Joseph Madri, Department of Pathology, Yale University School of Medicine.

Acid soluble type I collagen was prepared from calf skin and pepsin soluble type III collagen from human placental membranes according to the method of Burgeson et al. (1976).

Antibodies to type I and type III collagen were raised in rabbits and purified by immunoadsorbtion on affinity columns containing specific antigen (Nowack et al., 1976). The specificity of the antibodies was enhanced by cross-adsorbtion on affinity columns in which the cyanogen bromide activated Sepharose IV beads were linked to the other collagen types (Table 6). The titer of the primary antibodies was 1 to 100,000 by hemagglutination-inhibition (Figure 2).

The secondary antibody used was fluorescein-isothiocyanate conjugated sheep anti-rabbit IgG fraction (Hyland Laboratories).

Primary and secondary antibodies were stored at -60^{o} C, and diluted 1 to 1000 and 1 to 20, respectively, with phosphate buffered saline (PBS) prior to use.

TABLE 6: COLLAGEN TYPES USED IN AFFINITY COLUMNS FOR PURIFICATION OF TYPE-SPECIFIC ANTI-COLLAGEN ANTIBODIES*

Sera	Affinity Columns for Cross-adsorptions	Affinity Columns for Immunoadsorption
Anti type I	II, III, IV, V	I
Anti type II	I, III, IV, V	II
Anti type III	I, II, IV, V	III
Anti type IV	I, II, III, V	IV
Anti type V	I, II, III, IV	V

*From Madri and Furthmayr (1979b).

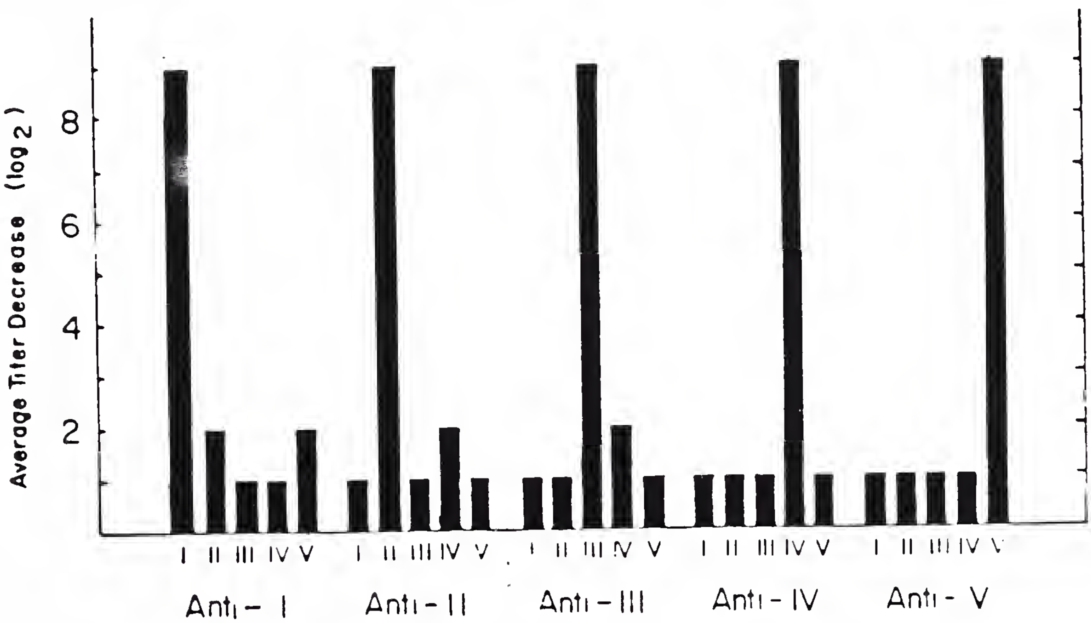


Figure 2. Hemagglutination-inhibition study of the specificity of collagen antibodies. Initial dilution of antibodies was 1:100. Concentration of collagens used as inhibitors was 2.5 micrograms. From Madri and Furthmayr (1979b).

2. Saphenous Veins

Thirteen saphenous veins were obtained from twelve patients undergoing initial coronary artery bypass surgery at Yale-New Haven Hospital, and from one patient undergoing a second revascularization procedure at the West Haven Veterans Administration Hospital. These patients represent a non-consecutive series of cases between September 30 and December 31, 1978. Characteristics of this group of patients are summarized in Table 7.

Six coronary artery bypass grafts were obtained from two patients undergoing a second CABG at Yale-New Haven Hospital (two grafts from each patient), and one patient having a second CABG at Yale-New Haven Hospital. Although one patient in this group received an internal mammary artery graft, the six grafts obtained were SV grafts. Clinical features of the three patients are summarized in Table 8.

All ungrafted veins were mechanically dilated with heparinized saline by the surgical team. The dilated vein was then measured and residual vein, usually about one to two centimeters, was provided for this work. Both ungrafted veins and excised bypass grafts were cut into multiple transverse sections, placed in a petri dish containing O.C.T. embedding medium (Lab-Tek), frozen in liquid nitrogen, and stored at -60^{o} C.

3. Staining Procedure

Slides were prepared by washing in 1% hydrochloric acid-absolute ethanol, drying with lint-free paper, coating with chicken egg albumin

TABLE 7: UNGRAFTED SAPHENOUS VEINS - PATIENT CHARACTERISTICS

Number of veins	13
Number of patients	13
Male	11
Female	2
Average age	60.2 years (46.8 to 78.0 years)
Male	57.9 years (46.8 to 72.2 years)
Female	72.4 years (66.7 and 78.0 years)

Histories of venous varicosities and/or evidence of venous stasis disease on physical examination were recorded in only one patient, a 47 year old male.

to prevent loss of tissue, and baking overnight at 60^{o} C.

Six micron frozen sections were cut from the O.C.T.-embedded veins on a cryostat. Multiple hematoxylin and cosin sections were prepared in order to assess the preservation of tissue architecture. Tissue deemed acceptable was then cut, with two sections placed on each slide. The slides were stored overnight at 4^{o} C.

The staining method is based on one that has been in routine use for immunofluorescent studies of kidney biopsy frozen sections at Yale-New Haven Hospital. Methods of this kind have been described in detail by Goldman (1968) and do not, in principle, differ essentially from the first accounts of indirect immunofluorescent methods (Weller and Coons, 1954; Mellors, Arias-Stella, et al., 1955). The technique as a whole derives from the work of Coons and co-workers (1942), who

TABLE 8: BYPASS GRAFTS - CLINICAL FEATURES

	Case 1	Case 2	Case 3
Age	52	58*	49
Sex	M	M	F
Initial Surgery	SV to RCA and L-CIRC	SV to RCA and L-CIRC; IMA to LAD	SV to RCA and L-CIRC
Risk Factors for ASCVD	AODM, hyper-lipidemia, FH and smoking	AODM, FH and smoking	Hyperlipidemia, FH, smoking
Interoperative Interval	60 months	48 months	32 months
Catheterization Prior to Second CABG	RCA graft occluded, L-CIRC graft patent	RCA graft patent, L-CIRC graft occluded, high grade IMA lesion	RCA and L-CIRC grafts occluded

* A segment of SV prepared for use as a repeat graft was obtained from this patient, and is included in the total in Table 8.
AODM - adult onset diabetes mellitus; FH - positive family history for ASCVD; LAD - left anterior descending artery; L-CIRC - left circumflex artery; RCA - right coronary artery; IMA - internal mammary artery.

described the use of impure fluorescein-isocyanate conjugated antibodies to type 3 pneumococcus to demonstrate the presence of that organism in infected mice. This direct method has largely given way to the convenience and flexibility afforded by the indirect technique.

Sections and staining materials were allowed to reach room temperature. Slides were fixed for ten minutes in acetone, and rehydrated for three minutes in PBS.

Primary antibody was applied for sixty minutes, and slides were washed for a total of nine minutes in three changes of PBS. Secondary antibody was then applied for forty minutes, and the slides washed as above. All staining was done in a covered moist box to prevent tissue dessication. The stained slides were then mounted with polyvinyl alcohol-glycerin and stored at 4^{o} C for one hour, after which they were ready for photomicrography.

4. Photomicrography

A Zeiss fluorescence microscope and camera were used, with a vertical illuminator using a super pressure mercury arc lamp as the light source. A Zeiss barrier filter was utilized which permitted the passage of excitation wavelengths ranging from 450 to 490 nanometers, and transmitted wavelengths greater than 520 nanometers. The excitation wavelength is ideal for work with fluorescein conjugates, as the absorption spectrum of fluorescein begins to rise at 420 nanometers and is maximal between 485 and 495 nanometers (Goldman, 1968). The transmitted spectrum permits easy recognition of fluorescein's sharp green fluorescence, as well as the yellow autofluorescence characteristic of elastic tissue (Hicks and Matthaei, 1955).

The extent of fluorescein fluorescence was assessed on an arbitrary 0 to 4+ scale for each section stained with anti-type I and anti-type III collagen antibodies.

Photomicrographs were obtained of all stained veins. Sections at 250X magnification were photographed with Kodak ASA 400 Ektachrome

film for slides, and developed at 400. Exposures were arbitrarily interrupted at three minutes.

RESULTS

A summary of the fluorescence noted in each specimen is presented in Table 9.

No remarkable pattern of distribution of fluorescence in the venous wall for each collagen type was noted. In general, specimens graded 3+ to 4+ displayed a diffuse, homogeneous fluorescence throughout the vein wall. Specimens graded 1+ and 2+ tended to have their fluorescence concentrated at the abluminal side of the vein wall.

Absence of type III collagen, as gauged by absence of specific fluorescence, was not observed in all 13 ungrafted saphenous veins, and the six bypass grafts. Type I collagen was not present in 4 of the 13 normal veins (30%) and 2 of 5 grafts (40%).

When both type I and type III collagen were present, fluorescence attributable to staining of type III collagen exceeded that due to type I collagen in 8 of 9 ungrafted veins (89%) and 3 of 3 grafts.

Maximal (4+) fluorescence was seen only in normal veins, 5 of 13 (38%) of which displayed 4+ fluorescence with respect to type III collagen.

The two grafts which had 2+ fluorescence with respect to type I collagen were angiographically demonstrated to be occluded, while the two grafts which failed to stain positively for type I collagen were shown to be patent at angiography (see Table 8).

Yellow autofluorescence suggestive of elastic tissue was not prominent in any section and when present took the form of wavy streaks diffusely distributed throughout the vein. A well developed

TABLE 9: FLUORESCENCE IN STAINED SPECIMENS

Ungrafted Veins

Age of Patient	Sex	Type I Collagen	Type III Collagen
57.0	M	1+	3+
63.0	M	3+	4+
58.0[1]	M	0	4+
66.7	F	3+	3+
59.0	M	0	2+
54.3	M	1+	4+
47.1[2]	M	1+	3+
58.8	M	3+	3+
61.0	M	0	3+
60.4	M	1+	2+
78.0	F	1+	4+
72.2	M	1+	4+
46.8	M	0	3+

[1]This patient's original bypass grafts were obtained during a second bypass procedure (case number 2).

[2]Superficial varicosities were noted in this patient.

Coronary Artery Bypass Grafts

	Type I Collagen	Type III Collagen
Case 1		
Graft to RCA	2+	3+
Graft to L-CIRC	0	3+

TABLE 9 (Cont'd)

	Type I Collagen	Type III Collagen
Case 2		
Graft to RCA	0	3+
Graft to L-CIRC	2+	3+
Case 3		
Graft to RCA	-	2+
Graft to L-CIRC	1+	3+

internal or external elastic lamina was not seen in any section.

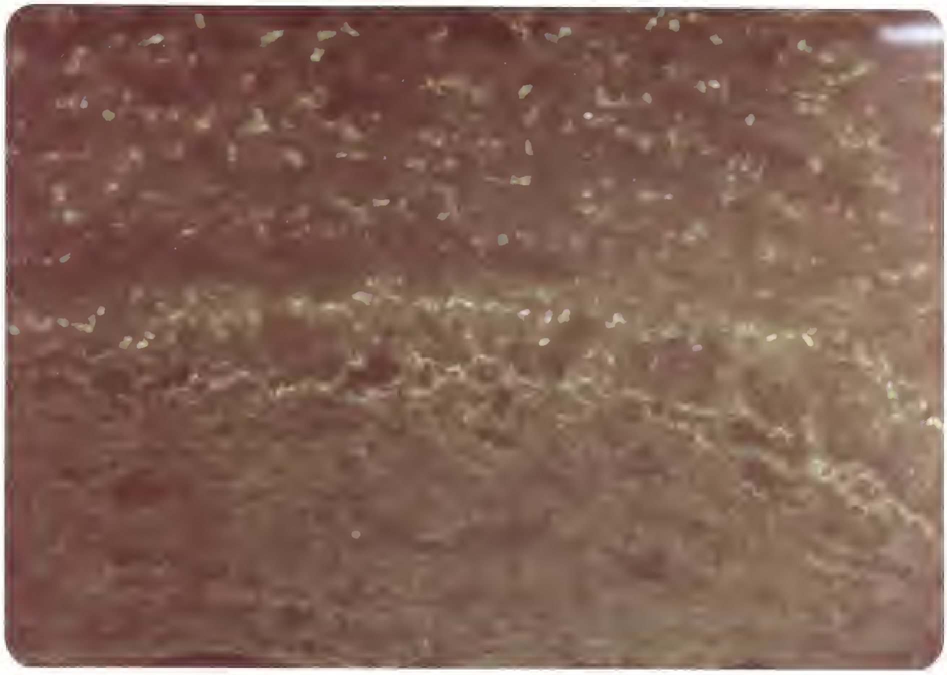
Dull green autofluorescence was seen at the cut edge of sections. This fluorescence might have been enhanced by the frozen section method of tissue processing.

The appropriate statistical test of significance for these data, Chi-square with one degree of freedom, was not applied because the small sample size precludes meaningful application of this method.

Examples of prominent type III and weak type I fluorescence in an ungrafted vein are presented in Figures 3 and 4. Type III predominance is also shown in the graft to the right coronary artery from case 2 (Figures 5 and 6), and appreciable fluorescence attributable to both collagen types are demonstrated in the graft to the left circumflex coronary artery of the same patient (Figures 7 and 8).

Figure 3. Ungrafted saphenous vein from a 57 year old man. Type I collagen. Lumen is below. Non-specific fluorescence is evident in wavy fragments scattered throughout the vein wall. This might represent elastin. 250X.

Figure 4. Same vein as in Figure 3. Type III collagen. Poor preservation of tissue architecture is typical of distended veins. 250X.



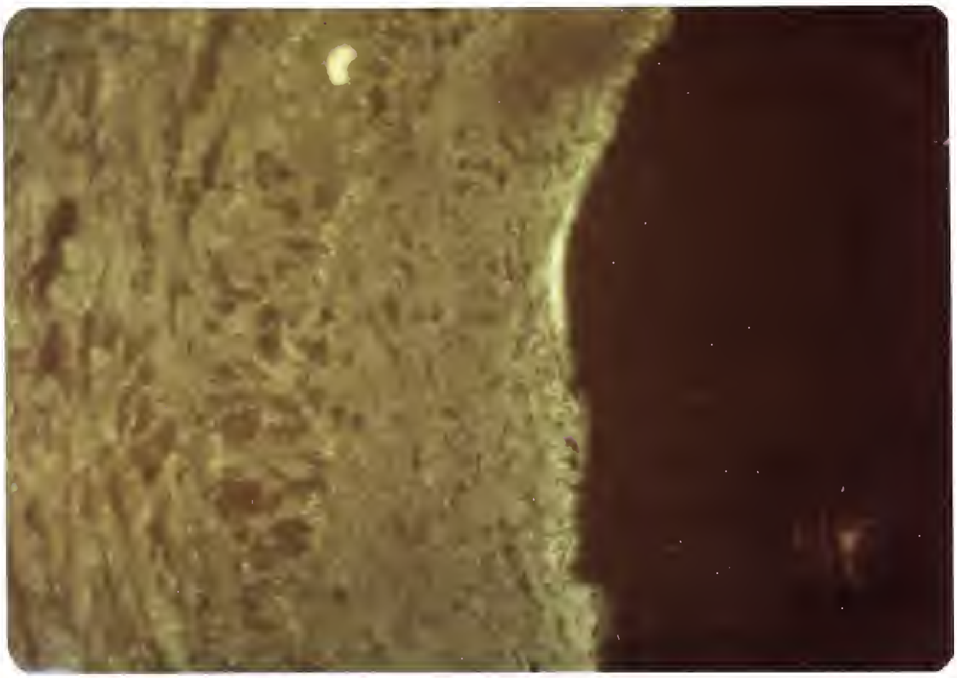
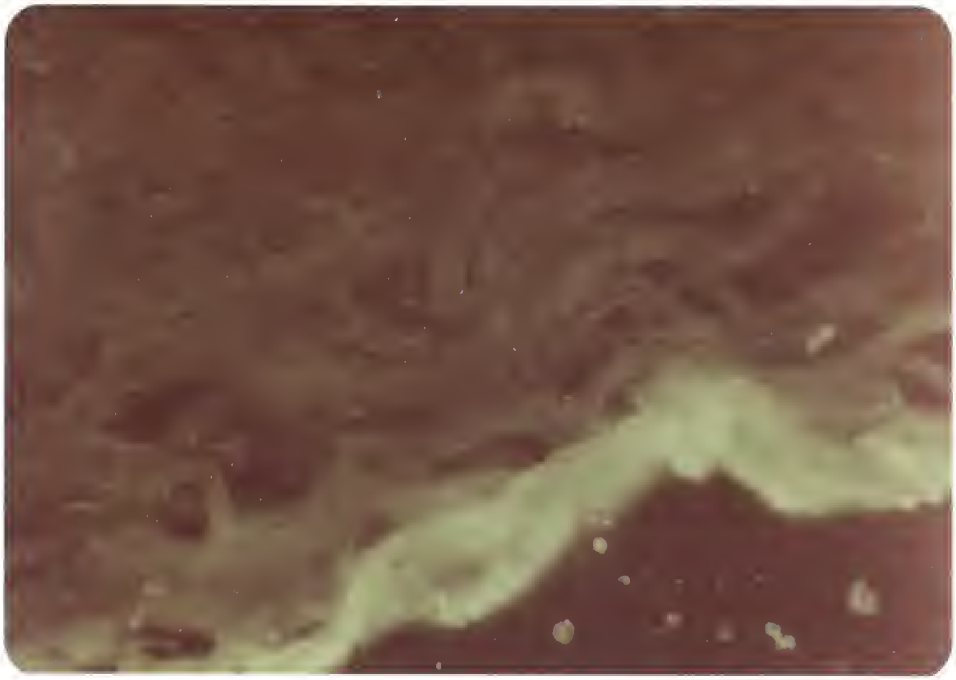


Figure 5. Saphenous vein bypass graft to right coronary artery (case 2). Type I collagen. Tissue is folded at luminal edge. 250X.

Figure 6. Same vein graft as in Figure 5. Type III collagen. Lumen is to the right. 250X.



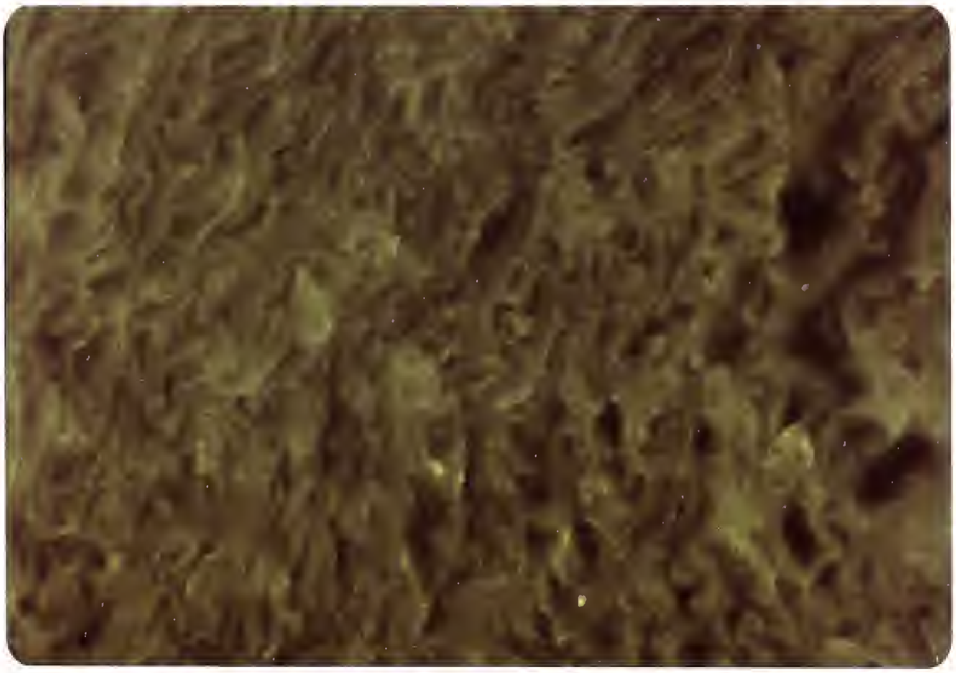
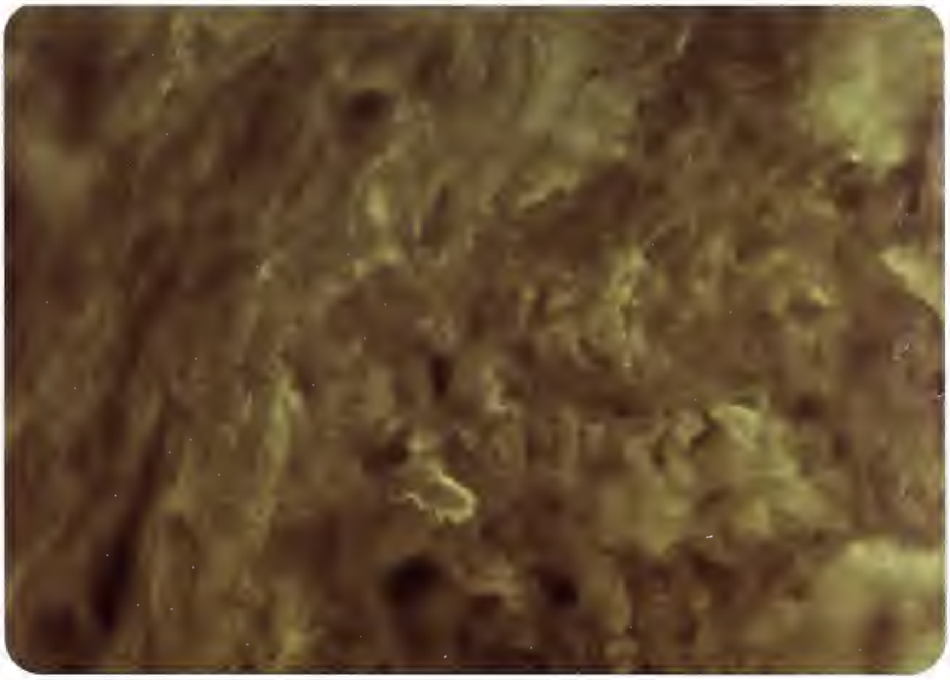
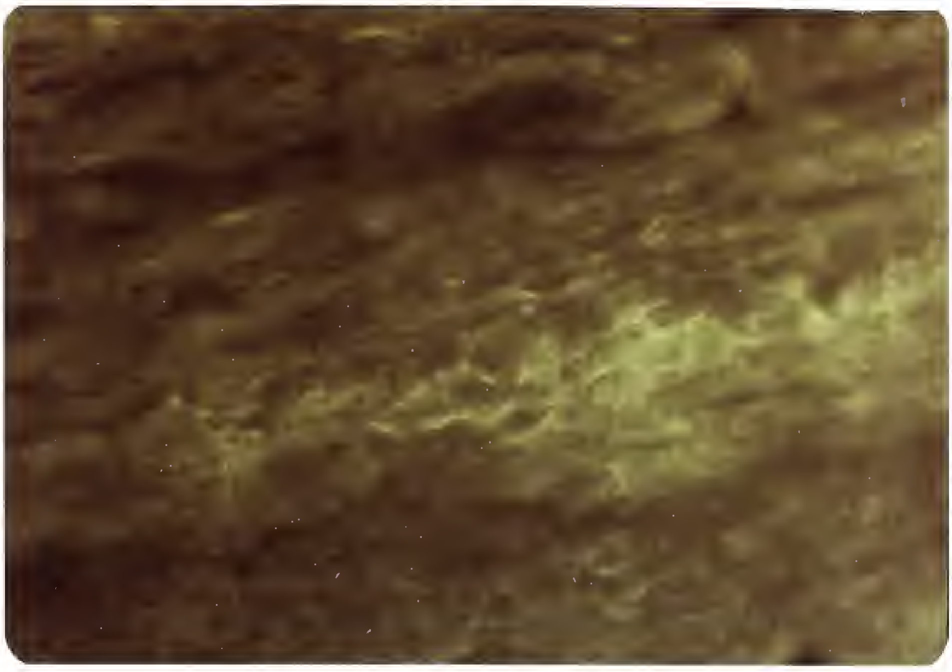


Figure 7. Saphenous vein bypass graft to left circumflex coronary artery (case 2). Type I collagen. Lumen is to the right. 250X.

Figure 8. Same graft as in Figure 7. Type III collagen. Lumen is below. 250X.





DISCUSSION

The relative quantities of type I and type III collagen in grafted saphenous veins demonstrated in this study do not permit the drawing of firm conclusions. It appears that type III collagen predominates in both ungrafted and grafted veins, with possibly more type III collagen in ungrafted veins. Remarkable differences in fluorescence due to type I collagen were not apparent.

A major difficulty of this analysis, aside from the limitations imposed by its qualitative nature, is the fact that only one of the six studied vein grafts was demonstrated to be widely patent at preoperative coronary angiography. The precise length of time for which the grafts were actually exposed to increases in transmural pressure cannot be determined. Observed changes might have differed if sections of graft were obtained proximal and distal to an obstruction. In addition, changes in collagen type might have been accentuated had normal veins been obtained from younger patients. The effects of age on collagen type in the vascular system have not been established.

Alterations in the ratio of type I to type III collagen has been demonstrated in human atherosclerotic plaques. By means of cyanogen bromide peptide analysis, McCullagh and Ballian (1975) found that normal human aortas contain 70% type III collagen and 30% type I collagen, while atherosclerotic aortas contain an average of 35% type III and 65% type I collagen.

This change in the preponderant collagen type introduces an important additional dimension to the knowledge which has accumulated in

experimental animals regarding overall alterations in collagen metabolism in atherosclerosis (Langner and Modrak, 1976; McCullagh and Erhart, 1974 and 1977) and hypertension (Spector et al., 1978). These studies have complemented the body of morphologic evidence reviewed above regarding the development of fibrosis in the vascular wall in those disease states. Increased collagen biosynthesis and/or turnover in the arterial wall accompany both hypertension and atherosclerosis. Whether such changes are accelerated in venous grafts has not been determined, but appears likely on morphologic grounds.

Changes in collagen type have been demonstrated in other disease states. A decrease in type III collagen (31% to 12-24%), with a relative increase in type I collagen, has been seen in lungs from patients with idiopathic pulmonary fibrosis as compared with controls (Seyer, Hutcheson and Kang, 1976). In this study, lung from a patient with pulmonary fibrosis and scleroderma, a condition in which a propagable increase in collagen biosynthesis by dermal fibroblasts has been demonstrated (Buckingham et al., 1978), did not display an alteration in collagen type.

Similarly, a decrease in type III collagen (47% to 18-34%) with a proportionate increase in type I collagen has been found in livers of patients with Laennec's cirrhosis, as compared with controls (Seyer, Hutcheson and Kang, 1977). Previous work by Rojkind and Martinez-Palomo (1976) indicating that normal liver contains 80% type I collagen while livers with Laennec's cirrhosis have increased quantities of both type I and III collagen, with both present in equal proportions, may have been affected by cross-contamination during the

isolation of the individual collagen types.

It is tempting to speculate that alterations in the ratio of type I to type III collagen, with possible increases in type I and decreases in type III collagen, might be characteristic of fibrotic processes in diverse settings. The perturbations which might affect specific collagen type biosynthetic and degradative pathways remain to be determined. The functional significance of alterations in collagen type are also unknown.

One indication of type III collagen's function in the vascular wall emerges from an immunofluorescent microscopic study of human arteries (Gay et al., 1975). Type III collagen was shown to be diffusely present throughout the arterial wall, but was primarily localized in the subendothelium. Type I collagen was less prominent in the abluminal side of the vessel wall, but was seen to be increased towards the adventitia. The predominance of type III collagen in the subendothelium suggested that this collagen type might be especially effective in the induction of platelet aggregation and thrombosis.

Which collagen type is most effective as a platelet aggregator is controversial. Acid solubilized human and chick type III collagen has been found to be more potent in causing platelet aggregation than type I collagen. This difference was reduced substantially when the collagens were treated with Na_2HPO_4 to induce fibrillogenesis -- an important finding, since native collagen is present in fibrillar form in the tissues (Barnes, Gordon and MacIntyre, 1976). Baillesen et al. (1975) found that fibrillar bovine type III collagen is a more powerful inducer of platelet aggregation than a similar preparation of

type I collagen. More recent work has indicated that the actual three dimensional configurations of the collagens is of greater consequence in determining platelet aggregation than individual differences in amino acid sequence among the collagen chains (Gordon, 1979). The future use of platelet collagen receptors, such as the fibronectin glycoproteins, as molecular probes may help to further clarify the nature of platelet-collagen interactions (Bensusan et al., 1978).

While evidence for polarity in the distribution of type I and III collagen in ungrafted and grafted saphenous veins was not obtained in this study, future work might delineate such changes, and relate them to the observed propensities of early and late grafts to become thrombosed.

An additional indication of the possible function of type III collagen in the vascular wall comes from an investigation of the molecular basis of the Ehlers-Danlos Syndrome, Type IV. Joint hyperextensibility is not as prominent in this disorder as in the other types making up the spectrum of the Ehlers-Danlos Syndrome. Rather, easy bruisability and death secondary to arterial rupture and gastrointestinal perforation are frequent findings (Pinnell, 1978). Patients with this disease have been shown to have marked reductions in aortic type III collagen, and their skin fibroblasts appear capable solely of synthesizing type I collagen (Pope et al., 1975).

Changes in the composition of the collagens occur in fibrotic processes in parenchymal organs and in atherosclerosis. Type III collagen may be of prime importance in the maintenance of overall

structural integrity in the vascular wall. Collagen type and distribution may affect a blood vessel's susceptibility towards thrombosis. At this juncture, very little can be concluded about the pathological significance of collagen polymorphism.

BIBLIOGRAPHY

Abbott WM, Wieland S and Austen WG (1974). Structural changes during the preparation of autogenous vein grafts. Surgery 76: 1031

Allard C, Ruscito O and Goulet C (1972). The influence of serum triglycerides on the fate of aortocoronary vein grafts. Can Med Assoc J 107: 213

Balleisen L, Gay S, Marx R and Kuhn K (1975). Comparative investigation of the influence of human and bovine collagen types I, II and III on the aggregation of human platelets. Klin Wschr 57: 903

Barboriak JJ, Pintar K and Korns ME (1974). Atherosclerosis in aortocoronary vein grafts. Lancet 2: 621

Barboriak JJ, Batayias GE, Pintar K and Korns ME (1976a). Pathological changes in surgically removed aortocoronary vein grafts. Ann Thorac Surg 21: 524

Barboriak JJ, Van Horn DL, Pintar K, Batayias GE and Korns ME (1976b). Scanning electron microscopic study of human veins and aorta-coronary vein grafts. J Thorac Cardiovasc Surg 71: 673

Barboriak JJ, Batayias GE, Pintar K, Thu TM, Van Horn DL and Korns ME (1977). Late lesions in aorta-coronary vein grafts. J Thorac Cardiovasc Surg 73: 596

Barboriak JJ, Pintar K, Van Horn DL, Batayias GE and Korns ME (1978). Pathologic findings in the aortocoronary vein grafts: a scanning electron microscopic study. Atherosclerosis 29: 69

Barnes MJ, Gordon JL and MacIntyre DE (1976). Platelet aggregating activity of type I and type III collagens from human aorta and chicken skin. Biochem J 160: 647

Batayias GE, Barboriak JJ, Korns ME and Pintar K (1977). The spectrum of pathological changes in aortocoronary saphenous vein grafts. Circulation (supp II) 56: II-18

Beebe HG, Clark WF and DeWeese JA (1970). Atherosclerotic changes occurring in an autogenous venous arterial graft. Arch Surg 101: 85

Bensusan HB, Koh TL, Henry KG, Murray BA and Culp LA (1978). Collagen receptor might be fibronectin. Proc Nat Acad Sci 75: 5864

Berk PD, Popper H, Krueger GRF et al. (1979). Veno-occlusive disease of the liver after allogeneic bone marrow transplantation. Ann Int Med 90: 158

Bloom W and Fawcett DW (1975). A Textbook of Histology, 10th edition. Philadelphia, WB Saunders Company, pp. 409-413

Bond MG, Hostetler JR, Karayannacos PE, Geer JC, Vasco JS (1976). Intimal changes in arteriovenous bypass grafts: effects of varying the angle of implantation at the proximal anastamosis and of producing stenosis in the distal runoff arteries. J Thorac Cardiovasc Surg 71: 907

Bornstein P (1974). The Biosynthesis of Collagen. Ann Rev Biochem 43: 567

Bourassa MG, Lesperance J, Campeau L and Simard P (1972). Factors influencing patency of aortocoronary vein grafts. Circulation 45-46 (suppl I): I-79

Breyer RH, Spray TL, Kastl DG and Roberts WC (1976). Histologic changes in saphenous vein aorta-coronary bypass grafts. J Thorac Cardiovasc Surg 72: 916

Brody WR, Angell WW and Kosek JC (1972). Histologic fate of the venous coronary artery bypass in dogs. Am J Path 66: 111

Brody WR, Kosek JC and Angell WW (1972). Changes in vein grafts following aorta-coronary bypass induced by pressure and ischemia. J Thorac Cardiovasc Surg 64: 847

Brook WH (1977). Vasa vasorum of veins in dog and man. Angiology 28: 351

Buckingham RB, Prince RK, Rodnan GP and Taylor F (1978). Increased collagen accumulation in dermal fibroblasts cultures from patients with progressive systemic sclerosis (scleroderma). J Lab Clin Med 92: 5

Buda JA, Weber CJ, McAllister FF, Voorhess AB (1976). Factors influencing patency of femeropopliteal artery bypass grafts. Am J Surg 132: 8

Bulkley BH and Hutchins GM (1977). Accelerated "atherosclerosis" - a morphologic study of 97 saphenous vein coronary artery bypass grafts. Circulation 55: 1

Bulkley BH and Hutchins GM (1978). Pathology of coronary artery bypass graft surgery. Arch Path Lab Med 102: 273

Burgeson RE, El Adli F, Kaitila II and Holister DW (1976). Fetal membrane collagens: identification of two new collagen alpha chains. Proc Nat Acad Sci 73: 2579

Caro CG, Fitz-Gerald JM and Schroter RC (1969). Arterial wall shear and distribution of early atheromas in man. Nature 223: 1159

Carrington C and Liebow AA (1970). Pulmonary veno-occlusive disease. Huamn Path 1: 322

Chung E and Miller EJ (1974). Collagen polymorphism: characterization of molecules with chain composition $[\alpha 1(\mathrm{III})]_3$ in human tissues. Science 183: 1200

Coons AH, Creech HJ, Jones NJ and Berliner E (1942). The demonstration of pneumococcal antigens in tissues by the use of fluorescent antibody. J Immunol 45: 159

Crawford T (1977). "Blood and Lymphatic Vessels" in Pathology, 7th ed., WAD Anderson and JM Kissane, eds. St. Louis, CV Mosby Company, pp. 883-884

Dean RH, Wilson JP, Burko H and Foster JH (1974). Saphenous vein aortorenal bypass grafts: serial ateriographic study. Ann Surg 180: 469

DeBakey ME, Jordan GL, Abbott JP, Halpert B and O'Neal RM (1964). The fate of dacron vascular grafts. Arch Surg 89: 757

DeWanjee, Fuster V, Kaye MP and Josa M (1978). Imaging platelet deposition with Indium-111 labelled platelets in coronary artery bypass grafts in dogs. Mayo Clinic Proc 53: 327

Dixon KC (1961). Deposition of globular lipid in arterial cells in relation to anoxia. Am J Path 39: 65

Duguid JB (1946). Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. J Path Bact 58: 207

Duguid JB (1948). Thrombosis as a factor in the pathogenesis of aortic atherosclerosis. J Path Bact 60: 57

Ejrup B, Hiertonn T and Moberg A (1961). Atheromatous changes in autogenous vein grafts - functional and anatomical aspects (a case report). Acta Chir Scand 121: 211

Farry J, Hammond GL, Cohen LS and Wolfson S (1976). Development of atherosclerosis in a saphenous vein bypass graft. Ann Int Med 85: 478

Faulkner SL, Fisher RD, Conkle DM, Page DL and Bender HW (1975). Effects of blood flow rate on subendothelial proliferation in venous autografts used as arterial substitutes. Circulation 51-52 (supp I): I-163

Favaloro RG (1968). Autograft replacement of severe segmental coronary artery occlusion: operative technique. Ann Thorac Surg 5: 334

Fishman J, Ryan G and Karnovsky M (1975). Endothelial regeneration in the rat carotid artery and the significance of endothelial denudation in the pathogenesis of myointimal thickening. Lab Invest 32: 339

Florey H (1960). Coronary Artery Disease. Brit Med J 2: 1329

Franklin KJ (1931). Further notes on the arrangement of the collagen fibers of veins, together with certain other observations on veins and on the venous return. J Anat 66: 602

Franklin KJ (1933). Observations on the venae cavae of certain mammals. J Anat 67: 382

Franklin KJ (1937). A Monograph on Veins. Springfield, Ill., Charles C Thomas, 410 pp.

Friedman M, Byers SO, St George S et al. (1962). Origin of lipid and cholesterol in experimental thromboatherosclerosis. J Clin Invest 41: 828

Friedman M (1963). Spontaneous atherosclerosis and experimental thromboatherosclerosis. Arch Path 76: 571

Furuse A, Klopp EH, Brawley RK and Gott VL (1972). Hemodynamics of aorta-to-coronary bypass: experimental and analytical studies. Ann Thorac Surg 14: 283

Gay S, Balleisen L, Remberger K, Fietzek PP, Adelman BC and Kuhn K (1975). Immunohistochemical evidence for the presence of collagen type III in human arterial walls, arterial thrombi, and in leukocytes incubated with collagen in vitro. Klin Wschr 53: 899

Geiringer E (1949). Venous atheroma. Arch Path 48: 410

Gessner FB (1973). Hemodynamic Theories of Atherogenesis. Circ Res 33: 259

Gillman T, Penn J, Bronks D and Roux M (1955). Abnormal elastic fibers: appearance in cutaneous carcinoma, irradiation injuries, and arterial and other degenerative connective tissue lesions in man. Arch Path 59: 733

Glagov S (1973). Mechanical stresses on vessels and the non-uniform distribution of atherosclerosis. Med Clin North Am 57: 63

Goldman M (1968). Fluorescent Antibody Methods. New York and London, Academic Press, 303 pp.

Grondin CM, Meere C, Castonguay Y, Lepage G and Grondin P (1971a). Progressive and late obstruction of an aortocoronary bypass graft. Circulation 43: 698

Grondin CM, Lepage G, Castonguay Y, Meere C and Grondin P (1971b). Aortocoronary bypass graft: initial flow through graft and early post-operative patency. Circulation 44: 815

Gross H and Handler B (1939). Sclerosis of the superior vena cava in chronic congestive heart failure. Arch Path 28: 22

Gross J (1974). "Collagen Biology: Structure, Degradation and Disease" in The Harvey Lectures, Series 68: 351

Haimovici H and Maier N (1971). Experimental canine atherosclerosis in autogenous abdominal aortic grafts implanted into the jugular vein. Atherosclerosis 13: 375

Haimovici H and Maier N (1974). Autogenous vein grafts in experimental canine atherosclerosis. Arch Surg 109: 95

Ham, AW (1974). Histology, 7th ed. Philadelphia, JB Lippincott Company, pp. 573-579

Hamaker WK, O'Connell TJ and Gomez AC (1972). Subintimal obliterative proliferation in saphenous vein grafts: a cause of early failure of aorta to coronary artery bypass grafts. Ann Thorac Surg 13: 488

Harrison LH (1976). Historical aspects in the development of venous autografts. Ann Surg 183: 101

Haust MD, More RH and Movat HZ (1960). The role of smooth muscle cells in the fibrogenesis of arteriosclerosis. Am J Path 37: 377

Hauswirth L and Eisenberg AA (1931). Disseminated venofibrosis (phlebosclerosis): its clinicopathological significance. Arch Path 11: 857

Heath D, Scott O and Lynch J (1971). Pulmonary veno-occlusive disease. Thorax 26: 663

Hicks JD and Matthaei E (1955). Fluorescence in histology. J Path Bact 70: 1

Hostetler JR, Karayannacos PE, Williams RA et al. (1976). Lessening of intimal thickening in arteriovenous grafts (abstract). Am J Cardiol 37: 144

Hurst JW, King SB, Logue RB et al. (1978). Value of coronary bypass surgery - controversies in cardiology, part I. Am J Cardiol 42: 308

Hutchins GM and Bannayan GA (1971). Development of endocardial fibroelastosis following myocardial infarction. Arch Path 91: 113

Hutchins GM and Ostrow PT (1976). The pathogenesis of the two forms of hypertensive pulmonary vascular disease. Am Heart J 92: 797

Kampmeier OF and Birch C La F (1927). Origin and development of venous valves, with particular reference to the saphenous district. Am J Anat 38: 451

Karayannacos PE, Hostetler JR, Bond MG, Kakos GS, Williams RA, Kilman JW and Vasko JS (1978). Late failure in vein grafts: mediating factors in subendothelial fibromuscular hyperplasia. Ann Surg 187: 183

Kennedy JH, Wieting DW, Hwang NHC et al. (1974). Hydraulic and morphologic study of fibrous intimal hyperplasia in autogenous saphenous vein grafts. J Thorac Cardiovasc Surg 67: 805

Kern WH, Dermer GB and Lindesmith GG (1972). The intimal proliferation in coronary saphenous vein grafts: light and electron microscopic studies. Am Heart J 84: 771

King P and Royle JP (1972). Autogenous vein grafting in atheromatous rabbits. Cardiovasc Res 6: 627

Klotz O, Permar HH and Guthrie CC (1923). End results of arterial transplants. Ann Surg 78: 305

Koelle GB (1975). "Neurohumoral transmission and the autonomic nervous system" in The Pharmacological Basis of Therapeutics, 5th ed., LS Goodman and A Gilman, eds. New York, MacMillan Publishing Company, Inc., pp. 404-409

Koontz TJ and Stansel HC (1972). Factors influencing patency of the autogenous vein-femeropopliteal bypass graft: an analysis of 74 cases. Surgery 71: 753

Langner RO and Modrak JB (1976). Collagen metabolism during the early stages of cholesterol-induced atherogenesis in rabbits. Blood Vessels 13: 257

Langou RA, Wiles JC and Cohen LS (1978). Coronary surgery for unstable angina pectoris: incidence and mortality of perioperative myocardial infarction. Brit Heart J 40: 767

Larson RM, Hagen P-O and Fuchs JCA (1974). Lipid biosynthesis in arteries, veins and vein grafts (abstract). Circulation 50 (suppl III): III-139

Lawrie GM, Lie JT, Morris GC and Beazley HL (1976). Vein graft patency

and intimal proliferation after aortocoronary bypass: early and long term angiopathologic correlations. Am J Cardiol 38: 856

Lesperance J, Bourassa MG, Biron P, Campeau L and Saltiel J (1972). Aorta to coronary artery saphenous vein grafts: preoperative angiographic criteria for successful surgery. Am J Cardiol 30: 459

Lesperance J, Bourassa MG, Saltiel J, Campeau L and Grondin CM (1973). Angiographic changes in aortocoronary vein grafts: lack of progression beyond the first year. Circulation 48: 633

Lev M and Saphir O (1951). Endophlebohypertrophy and phlebosclerosis. Arch Path 41: 154

Li P-L (1940). Adaptations in veins to increased intravenous pressure, with special reference to the portal system and inferior vena cava. J Path 50: 121

Lie JT, Lawrie GM and Morris GC (1977). Aortocoronary bypass saphenous vein graft atherosclerosis: anatomic study of 99 vein grafts from normal and hyperlipoproteinemic patients up to 75 months postoperatively. Am J Cardiol 40: 906

Ludbrook J (1962). Functional aspects of the veins of the leg. Am Heart J 64: 706

Ludbrook J (1966). Aspects of venous function in the lower limbs. Springfield, Ill., Charles C Thomas, 137 pp.

Madri JA and Furthmayr H (1979a). Isolation and tissue localization of type AB_2 collagen from normal lung parenchyma. Am J Path 94: 323

Madri JA and Furthmayr H (1979b). Collagen polymorphism in the lung: an immunochemical study of pulmonary fibrosis. Human Path (in press)

Malone PC and Morris CJ (1978). The sequestration and margination of platelets and leucocytes in veins during conditions of hypokinetic and anaemic anoxia: potential significance in clinical post-operative venous thrombosis. J Path 125: 119

Marti M-C, Bouchardy B and Cox JN (1971). Aortocoronary by-pass with autogenous saphenous vein grafts: histopathological aspects. Virchows Arch Path Anat 352: 1971

Mayne R, Vail MS and Miller EJ (1978). Characterization of the collagen chains synthesized by cultured smooth muscle cells derived from rhesus monkey thoracic aorta. Biochem 17: 446

McCabe M, Cunningham GJ, Wyatt AP, Rothnie NG and Taylor GW (1967). A histological and histochemical examination of autogenous vein grafts. Br J Surg 54: 147

McCann RL, Larson RM, Mitchener JS, Fuchs JCA and Hagen P-O (1979). Intimal thickening and hyperlipidemia in experimental primate vascular autografts. Ann Thorac Surg 189: 62

McIntosh HD and Garcia JA (1978). The first decade of aorto-coronary bypass grafting: 1967-1977 - a review. Circulation 57: 405

McCullagh KA and Balian G (1975). Collagen characterization and cell transformation in human atherosclerosis. Nature 258: 73

McCullagh KG and Erhart LA (1974). Increased arterial collagen synthesis in experimental canine atherosclerosis. Atherosclerosis 19: 13

McCullagh KG and Erhart LA (1977). Enhanced synthesis and accumulation of collagen in cholesterol-aggravated pigeon atherosclerosis. Am J Path 26: 341

Mellors RC, Arias-Stella J, Siegel M and Pressman D (1955). Analytical pathology II - histopathologic demonstration of glomerular-localizing antibodies in experimental glomerulo-nephritis. Am J Path 31: 687

Miller EJ and Lunde LG (1973). Isolation and characterization of the cyanogen bromide peptides from the α1(II) chain of bovine and human cartilage collagen. Biochem 12: 3153

Miller EJ and Matukas VJ (1969). Chick cartilage collagen: a new type of α1 chain not present in bone or skin of the species. Proc Nat Acad Sci 64: 1264

Miller EJ and Matukas VJ (1974). Biosynthesis of collagen: the biochemist's view. Fed Proc 33: 1197

Moschcowitz E (1959). Pathogenesis of phlebosclerosis - I. Phlebosclerosis of the portal vein. Arch Path 68: 1959

Moschcowitz E (1960). The pathogenesis of phlebosclerosis - III. Phlebosclerosis of the inferior vena cava. Ann Int Med 52: 723

Moschcowitz E (1962). Studies in phlebosclerosis - IV. Phlebosclerosis of the pulmonary veins. Am J Cardiol 10: 836

Moschcowitz E (1964). Studies in phlebosclerosis - VI. The immunity from phlebosclerosis in the coronary vein. Am J Cardiol 13: 495

Moschcowitz E and Strauss L (1964). Studies in phlebosclerosis - V. Phlebosclerosis of the inferior vena cava and portal vein in an infant 23 days old. Arch Path 77: 445

Mustard JF, Murphy EA, Roswell HC and Downe HG (1962). Factors influencing thrombus formation in vivo. Am J Med 33: 621

Nicholson BB (1927). Varicose veins. Arch Surg 15: 351

Nowack H, Gay S, Wick G, Becker U and Timpl R (1976). Preparation and use in immunohistology of antibodies specific for type I and type III collagen and procollagen. J Immunol Meth 12: 117

Orkin RW, Gehron P, McGoodwin EB et al. (1977). A murine tumor producing a matrix of basement membrane. J Exp Med 145: 204

Payan HM and Gilbert EF (1968). Micronodular phlebosclerosis. Am Heart J 75: 428

Payan HM and Gilbert EF (1972). Interrupted eccentric longitudinal muscle fibers of the kidney and adrenal veins. Am Heart J 84: 76

Penn I, Schenk E, Rob C, DeWeese J and Schwartz S (1965). Evaluation of the development of athero-arteriosclerosis in autogenous venous grafts inserted into the peripheral arterial system. Circulation 31-32 (suppl I): I-192

Perez-Tamayo R (1978). Pathology of collagen degradation. Am J Path 92: 509

Piez KA, Eigner EA and Lewis MS (1963). The chromatographic separation and amino acid composition of the subunits of several collagens. Biochem 2: 58

Pinnell SR (1978). "Disorders of collagen" in The Metabolic of Inherited Disease, 4th ed., JB Stanbury, JB Wyngaarden and DS Frederickson, eds. New York, McGraw Hill Book Company, pp. 1366-1394

Pintar K, Barboriak JJ, Johnson D and Eddy D (1978). Atherosclerotic aneurysm in aortocoronary vein grafts. Arch Path Lab Med 102: 287

Pope FM, Martin OR, Lichtenstein JR, Penttinen R, Gerson B, Rowe DW and McKusick VA (1975). Patients with Ehlers-Danlos Syndrome Type IV lack type III collagen. Proc Nat Acad Sci 72: 1314

Ramos JR, Berger K, Mansfield PB and Sauvage LR (1976). Histologic fate and endothelial changes of distended and non-distended vein grafts. Ann Surg 183: 205

Rauterberg J and Kuhn K (1971). Acid soluble calf skin collagen - characterization of the peptides obtained by cyanogen bromide cleavage of its $\alpha 1$-chain. Eur J Biochem 19: 398

Reich NE (1942). Primary portal phlebosclerosis. Arch Int Med 69: 117

Rhodin JAG (1968). Ultrastructure of mammalian venous capillaries, venules and small collecting veins. J Ultrastr Res 25: 452

Rhodin JAG (1974). Histology: A Text and Atlas. New York, Oxford University Press, pp. 358-368

Rojkind M and Martinez-Palomo A (1976). Increase in type I and type III collagens in human alcoholic liver cirrhosis. Proc Nat Acad Sci 73: 539

Roscher AA and Kern WH (1977). Aortocoronary bypass from the pathologist's point of view: a clinicopathologic study of 240 cases with 26 autopsies. Int Surg 62: 282

Ross R and Glomset JA (1973). Atherosclerosis and the arterial smooth muscle cell. Science 180: 1332

Ross R, Glomset J and Harker L (1977). Response to injury and atherogenesis. Am J Path 86: 675

Rossiter RJ, Brody WR, Kosek JC, Lipton MJ and Angell WW (1974). Internal mammary artery versus autogenous vein for coronary artery bypass graft. Circulation 50: 1236

Sako Y and Varco RL (1962). Ten year observations on autologous pericardial and venous grafts in the thoracic aorta: an experimental study. Surgery 51: 465

Seides SF, Borer JS, Kent KM, Rosing DR, McIntosh CL and Epstein SE (1978). Long-term anatomic fate of coronary artery bypass grafts and functional status of patients after operation. New Eng J Med 298: 1213

Seyer JM, Hutcheson ET and Kang AH (1976). Collagen polymorphism in idiopathic pulmonary fibrosis. J Clin Invest 57: 1498

Seyer JM, Hutcheson ET and Kang AH (1977). Collagen polymorphism in normal and cirrhotic liver. J Clin Invest 59: 241

Simionescu M, Simionescu N and Palade G (1975). Segmental differentiation of cell junctions in the vascular endothelium: the microvasculature. J Cell Biol 67: 863

Simionescu M, Simionescu N and Palade G (1976). Segmental differentiation of cell junctions in the vascular endothelium: arteries and veins. J Cell Biol 68: 705

Spector S, Coshima A, Iwatsuki K, Fuller G, Cardinale G and Udenfriend S (1978). Increased vascular collagen biosynthesis by hypertension and reversal by antihypertensive drugs. Blood Vessels 15: 176

Spray TL and Roberts WC (1977). Status of the grafts and the native coronary arteries proximal and distal to coronary anastamotic sites of aortocoronary bypass grafts. Circulation 55: 741

Stanley JC, Ernst CB and Fry WJ (1973). Fate of 100 aorto-renal vein grafts: characteristics of late graft expansion, aneurysmal dilatation and stenosis. Surgery 74: 931

Stehbens WE (1974). The ultrastructure of the anastamosed vein of experimental arteriovenous fistulas in sheep. Am J Path 76: 377

Stehbens WE and Karmody AM (1975). Venous atherosclerosis associated with arteriovenous fistula for hemodialysis. Arch Surg 110: 176

Stein AA, Rosenblum I and Leather R (1966). Intimal sclerosis in human veins. Arch Path 81: 548

Stewart GJ, Ritchie WGM and Lynch PR (1974). Venous endothelial damage produced by massive sticking and migration of leukocytes. Am J Path 74: 507

Storm FK, Gierson ED, Sparks FC and Barker WF (1975). Autogenous vein bypass grafts: biological effects of mechanical dilatation and adventitial stripping in dogs. Surgery 77: 261

Stuart M and Magarey FR (1960). The great veins in venous hypertension. J Path Bact 79: 319

Szilagyi DE, Elliot JP, Hageman JH, Smith RF and Dall'olmo CA (1973). Biological fate of autogenous vein implants as arterial substitutes - clinical, angiographic and histopathologic observations in femeropopliteal operations for atherosclerosis. Ann Surg 178: 232

Tedeschi C (1941). Fibromuscular thickening in the walls of veins adjacent to arteries. Anat Rec 79: 243

Texon M, Imparato AM and Lord JW (1960). The hemodynamic concept of atherosclerosis: the experimental production of hemodynamic arterial disease. Arch Surg 80: 47

Ts'ao C-L and Spaet TH (1967). Ultramicroscopic changes in the rabbit inferior vena cava following partial constriction. Am J Path 51: 789

Ts'ao C-L, Glagov S and Kelsey BF (1971). Structure of mammalian portal vein: post-natal establishment of two mutually perpendicular medial muscle zones in the rat. Anat Rec 171: 457

Uitto J and Lichtenstein JR (1976). Defects in the biochemistry of collagen in diseases of connective tissue. J Invest Derm 66: 59

Unni KK, Kottke BA, Titus JL, Frye RL, Wallace RB and Brown AL (1974). Pathologic changes in aortocoronary saphenous vein grafts. Am J Cardiol 34: 526

Vlodaver Z and Edwards JE (1971). Pathologic changes in aortic-coronary arterial saphenous vein grafts. Circulation 44: 719

Wagner FB and Herbut PA (1949). Etiology of primary varicose veins: histologic study of 100 saphenofemoral junctions. Am J Surg 78: 876

Walker JA, Friedberg HD, Flemma RJ and Johnson WD (1972). Determinants of angiographic patency of aortocoronary vein bypass grafts. Circulation 45-46 (suppl I): I-86

Walton KW (1975). Pathogenic mechanisms in atherosclerosis. Am J Cardiol 35: 542

Weller TH and Coons AH (1954). Fluorescent antibody studies with agents of varicella and herpes zoster propagated in vitro. Proc Soc Exp Biol Med 86: 789

Whiteley HJ (1953). Atheroma of the portal vein. J Path Bact 66: 563

Wilens SL and McCluskey RT (1952). The comparative filtration properties of excised arteries and veins. Am J Med Sci 224: 540

Wilson JB (1961). Changes in the portal and splenic veins in portal hypertension and their relation to splenomegaly. Gut 2: 310

Woolfe N (1961). The distribution of fibrin within the aortic intima: an immunohistochemical study. Am J Path 39: 521

World Health Organization (1958). Classification of atherosclerotic lesions: report of a study group. Technical Report Ser No. 143

Wyatt AP, Rothnie and Taylor GW (1964). The vascularization of vein grafts. Br J Surg 51: 378

Wyatt AP and Taylor GW (1966). Vein grafts: changes in the endothelium of autogenous free vein grafts used as arterial replacements. Br J Surg 53: 943

Wyatt AP and Gonzales (1969). Atheromatous lesions in arterialized vein grafts: an experimental study. Br J Surg 56: 193

Young JN, MacMillan JC, May IA, Iverson LIG and Ecker RR (1978). Internal configuration of saphenous-coronary anastamoses as studied by the cast-injection technique. J Thorac Cardiovasc Surg 75: 178

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS		DATE
Gary J. Price, M.D	Dept of Surgery LSOG 307	5/15/80
G J Price	" " " "	5/19/80